THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXXII JULY–AUGUST, 1956 NUMBER 4

THE APPLICATION OF AN INDUCED BRONCHIAL COLLATERAL CIRCULATION TO THE CORONARY ARTERIES BY CARDIOPNEUMONOPEXY

I. ANATOMICAL OBSERVATIONS *

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That the human heart can come to be independent of its usual source of blood at the orifices of the coronary arteries in the sinuses of Valsalva has been established.[1-4] This fact has encouraged attempts to induce a collateral circulation to the heart. Four principles have been employed:

1. The induction of vascular connections via transpericardial adhesions
   a. With the pericardial vessels, the expansion of which was abetted by mechanical trauma,[5] or the introduction of foreign material such as talc,[6,7] or asbestos[8-10]; or
   b. With the vessels of other organs such as the (1) omentum,[9,11] (2) lung,[12-17] (3) skin,[18] (4) intestine,[19] (5) muscle[20]
2. The intramyocardial implantation of a systemic artery such as the internal mammary artery[21,22] or splenic artery[23]
3. The restriction of venous outflow[24-26]
4. The reversal of flow in the coronary venous system by connecting the aorta to the coronary sinus.[27-30]

Three main effects of these various techniques have been noted:

(1) The new development of transpericardial vessels that come to connect peripheral branches of the coronary arteries with branches of the pericardial vessels, or of the arteries of other organs involved in the

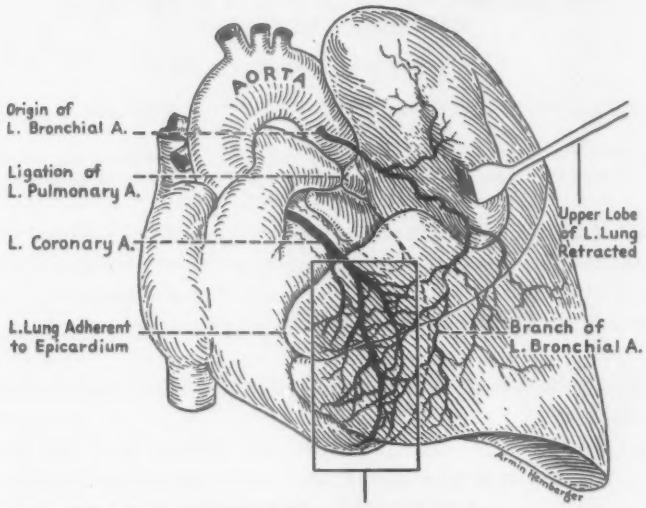
* Supported by a grant from the Office of Naval Research (N6ori–44, Task Order XI), and by the New Haven Heart Association.

Presented, in part, at the Fifty-second Annual Meeting of the American Association of Pathologists and Bacteriologists, Houston, Texas, April 7, 1955.

Received for publication, August 9, 1955.

cardiopexy, or of vessels introduced into the wall of the heart, as determined respectively by the various operations.

(2) The increase, or possibly induction, of intercoronary connections of precapillary size. It is remarkable that it is the development of these intercoronary connections that appears to be the lasting consequence of anastomosing the aorta to the coronary sinus, since the reversal of flow is said usually to come to a halt within 6 weeks.[27,31]



Text-figure 1. Schematic representation of cardiopneumonopexy after ligature of pulmonary artery. The bronchial arteries are shown traversing the left lung, and crossing adhesions between lung and heart, to anastomose with branches of the left coronary artery.

(3) The expansion of pre-existing minute vessels that connect the coronary arteries with extracardiac vessels. These have been described as normally accompanying the great vessels, especially the pulmonary veins, as they leave the heart to pass beyond the reflection of the pericardium. In man they have been well described.[32-35] We have observed, also, arteries derived from the descending thoracic aorta that enter the interatrial septum, after having yielded additional branches to other mediastinal structures. In the dog, although the coronary arterial system has been studied in detail,[36-38] descriptions of extracardiac connections of the coronary arteries have been meager and have been made only in the most general terms.

On the basis of available knowledge, and as briefly outlined in a preliminary report,[39] cardiopneumonopexy after ligature of the pulmo-

nary artery rather than simple cardiopneumonopexy as practiced by Lezius[13] would seem a logical method of supplementing the coronary arterial circulation. (1) A vast ingrowth of bronchial vessels from the aorta, far beyond that required for the metabolism of the lungs, has been demonstrated to occur after interruption of the pulmonary arteries.[40-44] (2) These vessels contain fully oxygenated blood at relatively high pressure. (3) Transpleural arterial connections are readily formed with such adjacent vessels as the intercostal and pericardiophrenic and these supplement the expanded bronchial collateral circulation. (4) The cardiopneumonopexy permits application of the collateral vascular bed over a large surface of the heart, to cover a portion of the right as well as the left ventricle. (5) Since the ostia of the coronary arteries in the sinuses of Valsalva and the sources of the major collateral vessels in the descending aorta are sufficiently far removed, a functional evaluation of collateral flow by means of appropriate catheterization methods is made possible.

After simple cardiopneumonopexy, Lezius[13] has demonstrated that anastomoses are formed between pulmonary arteries and the coronary vessels. Under these circumstances, however, desaturated blood from a pulmonary artery, if any blood at all, would gain entrance into the coronary vascular bed, and the pressure gradients would *a priori* seem less favorable than those existing after the establishment of a systemic collateral circulation to the lung. It is for this reason that ligature of the pulmonary artery and cardiopneumonopexy were performed at a single stage in the present investigation.

Methods

Operative Procedures

The operation was carried out under sodium pentobarbital (nembutal) anesthesia (35 mg. per kg.), using a Burns valve between an oxygen tank and an intratracheal cannula for inflation of the lungs under positive pressure. A midsternal incision at the first stage permitted access to all structures within the chest. Ligature of the left pulmonary artery was carried out within the pericardial sac. One of three additional procedures was completed at this stage.

A. Cardiopneumonopexy. For this purpose a defect was created in the pericardial sac just anterior to the left phrenic nerve, and the mesothelium of the pleural and epicardial surfaces to be apposed was coagulated with a silver nitrate stick. Lung and heart were sutured together, largely by means of mattress stitches taken through the anterior free margins of the lower and lingular lobes, and outer layers of the myocardium, with due care to avoid compression of major coronary arteries.

B. Wrapping of the trunk of the left main coronary artery with plastic, followed by cardiopneumonopexy. For this purpose the trunk of the left coronary artery was dissected free from the point of its emergence from the aorta to its major subdivi-

sions, and a strip of polyethylene approximately 2 mm. wide was wrapped about the trunk and tied in a square knot, as snugly as possible about the vessel, but without compression. Fixation of the knot was abetted by an encircling "F" silk tie. In some instances the trunk of the left coronary artery was so short that the bifurcation, or trifurcation, was embraced by the plastic. In two instances non-irritating polythene was used and in four instances irritating polythene impregnated with cetyl phosphate was employed.

C. Wrapping of the trunks of both coronary arteries in the manner just described, followed by cardiopneumonopexy.

D. Preparation for ligature of the coronary artery at a subsequent stage, employing the anterior descending branch; cardiopneumonopexy. Since preliminary work had demonstrated that an adequate cardiopneumonopexy made any attempt at subsequent ligature of the coronary artery extremely difficult, and since subsequent dissection partly defeated the purpose of the cardiopneumonopexy, a method was devised for obviating this difficulty. A simple loop of fine braided wire was passed beneath the vessel, and the free ends of the wire were confined within the lumen of a flanged segment of polyethylene, approximately 1 cm. long. The flange was lightly sewn to the epicardium at a distance on either side of the artery. The tubing successfully kept the lung from becoming adherent to the heart in the region of the pneumonopexy.

For animals in group D, the second stage, ligature of the left anterior descending branch, was carried out approximately 6 weeks after the first stage. The plastic cup containing the braided wire was easily exposed by an incision through the fourth left intercostal space. The cup was removed and the ligature was readily passed under the coronary artery, with the help of the previously placed guide-loop. The ligation of the coronary artery was performed by a two step method. The first step was to tie a constricting, but not occlusive, ligature. This was accomplished by tying down upon a number 19 needle placed next to the artery and then removing the needle. At the second step the ligature intended to be completely occlusive was tied. By this method all animals survived the operation without fibrillation.

Functional Studies

In 10 dogs in the present series, angiography was done by one of several methods to gain some knowledge of the hemodynamics within the induced collateral vascular system. In 5 animals, an attempt was made also to determine whether blood was flowing from the pulmonary collateral circulation to the heart and to estimate the volume of flow. For this purpose the aorta and appropriate branches and the coronary sinus were catheterized, and Evans blue dye was introduced at a point in the aorta just above the origins of the intercostal arteries that yield the major collateral branches to the lung. It was possible to repeat this procedure in each of the animals. The details of these methods are presented in Part II of this study,[57] together with appropriate controls. The animals were heparinized at the conclusion of the functional studies, and sacrificed with an overdose of nembutal. The various catheters were left *in situ* in order that their position might be confirmed at necropsy.

Preparation of Casts

Vinylite casts of the vessels of the heart and lung together were prepared in order to preserve their relationships. Since the details of these procedures have been reported elsewhere,[45,46] they will not be

repeated here. As a first step the axillary and carotid vessels were doubly ligated and the thorax was opened at the costochrondral junctions, taking care not to disturb adhesions that might be present on the left side. The internal mammary vessels of both sides were ligated, when it was established that they did not participate in the collateral circulation. The position of the catheters introduced for the functional studies was determined by palpation and inspection. The pulmonary artery, superior vena cava, and the inferior vena cava also were opened and the ligated conical termination of the proximal left pulmonary artery was inspected from within. Excess of blood was aspirated from the heart. The aorta was ligated just above the diaphragm, and transected approximately 1 cm. above the aortic valve ring. The excess of the still fluid heparinized blood was removed from the aorta by suction and the distal end of the vessel was cannulated. Air, water, air, acetone, and air were passed in that order under pressure into the aortic cannula. Vinylite was then introduced at 7 lbs. (chamber) pressure, followed by 28 per cent concentrated black-filled plastic. The coronary arteries were dissected as they emerge from the aorta, cannulas were introduced into their ostia in the sinuses, and tied into place. Seven per cent clear red plastic was introduced into these cannulas by syringe, followed by red 14 per cent plastic containing 5 per cent diatomaceous earth. After a series of reinjections over the next 30 minutes, to restore plastic shrunken by contact with fresh tissue, the ends of the polyethylene cannulas as they entered the coronary ostia were squeezed shut by means of metal skin clips (Michel). The polyethylene was then cut between the clip and the syringe. This step prevented leakage from the injected coronaries, or, in the opposite direction, from the aorta when this vessel was filled subsequently with a contrasting plastic mass. The latter consisted of 28 per cent filled green material. It was introduced by cannulating a pulmonary vein and injecting toward the left atrium. This filled the large pulmonary veins, atrium, ventricle, and usually a part of the aorta, the remaining short proximal segment of which had previously been occluded by cannulation. After setting of the plastic had taken place, this aortic cannula was reopened for the introduction of a small amount of additional thick green plastic. This then served to embed the metal clips, and the remaining ends of the coronary arterial cannulae. Thus the relations of the coronary vasculature were maintained.

After freeing the thorax and its contents by transecting the cervical and lowermost thoracic vertebrae, it remained to inflate the lungs in a vacuum chamber and to prepare a coarse bronchial cast with 28 per cent filled white plastic for the support of the vascular structures. Fixation of the cast of the left ventricle and proximal segment of aorta,

together with the attached coronary vessels, to the tracheobronchial cast was assured by the intertwining of the bronchi and pulmonary veins. The cast of the distal aorta and its branches was held in correct relationship to the bronchial cast by pinning them together with a straightened safety-pin before digestion of the tissue. After approximately 1 week to allow for hardening of the plastic, the entire thoracic cage with its contents was placed in concentrated hydrochloric acid to remove the tissue. The resulting cast permitted examination, from all aspects, of the vascular structures in their correct interrelationships.

Observations

General Observations

In the region of the cardiopneumonopexy the lingular portion of the upper lobe and a part of the lower were seen to be firmly fused to the anterior and left lateral surfaces of the heart. The serous surface of the epicardium continued over the adherent lung where a part of the pericardial sac persisted. In those animals in which the operation was a single stage procedure carried out through a midsternal incision, the lateral surface of the lower lobe was usually not adherent. Extensive adhesions were usually present in the re-operated animals. The lung was usually more shrunken than is the case after simple ligature of the pulmonary artery without pneumonopexy. This was, perhaps in part, the result of compression by a degree of stretching of the lung when it is sutured to the heart. In consequence of this tension there was also rotation of the heart on its long axis to the left. The ostium of the left coronary artery was thus somewhat more posterior than in the normal.

During the procedure of injecting air into the *distal* end of the transected ascending aorta in order to clear the smaller vessels of blood as has been described, there was in many of the experimental animals a remarkable bubbling of air from the coronary ostia as seen in the *proximal* stump of the transected aorta. This was immediate evidence of a collateral blood supply to the heart that connected the aorta distal to its ascending portion with the coronary arteries. Similarly, at the appropriate stages of the clearing and injection procedure, water and acetone, and, in 3 animals with the largest collateral circulation, 7 per cent black plastic were seen to issue from the coronary orifices in the sinuses of Valsalva. In dogs of group B, in which plastic had been wrapped about the coronary arteries, there was abundant fibrosis about the plastic, but probing revealed that the vessels had been little if at all constricted, although they were rigidly held to a certain caliber

where the plastic had been applied. This experience is similar to that of Neumann and co-workers.[18] Failure of significant constriction was confirmed by the appearance of the casts of the coronary arteries. The functional implications of this localized "rigidity" of the walls of these vessels are not known. Animals of groups A, B, and C may therefore tentatively be considered anatomically comparable.

It was disappointing, although instructive for future procedure, to find that only in 2 of the 9 animals of group D, in which attempts had been made to ligate the anterior descending coronary artery, had this vessel actually been permanently interrupted as demonstrated by the injection of plastic. In those in which braided wire had been used, it was still *in situ* as a loop at the point of ligature about the cast of the vessel. In a few animals, a slight indentation of the cast was seen here. Despite this apparent inadequacy of the ligature as seen at the end of the experiment, it is highly probable that at least a temporary interruption had been achieved. Those dogs in which ligature had been attempted but was not complete were classified in a separate group, D_1. This group also may be considered as perhaps comparable to groups A, B, and C.

Group D_2 represents the animals in which there was evidence that the descending coronary artery, or a large branch, had been permanently interrupted. In one of the 2 animals in which ligature had been successful, 3¾ weeks after the pneumonopexy (dog 321) there was palpable the thin scar of a large infarct in the distal portion of the left ventricle. In the other there was not an obvious infarct, although a thorough examination could not be performed because of the necessities of the injection procedure.

The Collateral Circulation in the Lung

As in previous observations, the bronchial arterial collateral circulation was found occasionally to be well expanded at 2½ months (group A, dog 291); and after 6 months in almost all of the dogs a plexus of vessels, each exceeding a diameter of 50 μ, extended to the terminal bronchioles, and usually there was also retrograde injection of the pulmonary artery from the periphery. The level of origin of the main bronchial collateral arteries from the aorta has been summarized in Table IV. These observations indicate that the tip of the catheter to be used for introducing a test substance such as Evans blue dye into the bronchial circulation should be placed within the aorta above the fifth intercostal space (first aortic intercostal artery) during the performance of bronchial arteriography.

Collateral Circulation to the Heart

Three major sources of collateral blood supply to the coronary arteries were observed in various animals: transpleural collaterals, retrocardiac collaterals, intercoronary collaterals. These are correlated with the various operative groups and with the factor of time in Tables I to III. In some of the dogs all three sources of collateral circulation

TABLE I

Development of Collaterals to Myocardium after Ligature of Left Pulmonary Artery and Cardiopneumonopexy

Group A

Simple pneumonopexy

Dog no.	Interval: pneumonopexy to sacrifice	Extent of pulmonary collaterals	Extent of cardiac collaterals		
			Transpleural	Intercoronary	Retrocardiac
	mos.				
290	1¾	++	+++	0	+
312	1¾	+	0	0	0
311	2¼	+	0	0	0
332	2¼	+	++	0	0
291	2½	+++	+++	0	0
292	3	+++	+++	0	+++
310	3	+	0	0	0
298	13½	++++	+++	0	++

Key (pulmonary collaterals):
+ = Slight expansion of bronchial arterial trunks at source.
++ = Moderate expansion to proximal bronchi only.
+++ = Filling to distal bronchi.
++++ = Filling of fine vessels about distal bronchi and retrograde injection of pulmonary arteries.

were present in varying degrees. In others there was only one. In none was there failure of some degree of collateral circulation to develop after cardiopneumonopexy. The various sources of the collateral blood supply are best discussed individually.

The Transpleural Collaterals

The earliest observations on transpleural collaterals were made in 2 animals, 7 weeks after the initial pneumonopexy (dogs 290 and 312). In one of these, plexuses of fine vessels had become established (Fig. 1). In the other, a dog ill from distemper and consequently sacrificed, there was no evidence of the development of collateral vessels as large as 50 μ in diameter although a pneumonopexy had become established. The transpleural collaterals directly joined branches of the coronary and bronchial arteries at many levels. The latter could be seen to wind spirally about small bronchioles in the region of the pneumonopexy

(Fig. 2). Most of the collateral vessels were connected directly with major branches of the coronary arteries, rather than with small distal twigs.

TABLE II

Development of Collaterals to Myocardium after Ligature of Left Pulmonary Artery and Cardiopneumonopexy

Dog no.	Interval: pneumonopexy to sacrifice	Extent of pulmonary collaterals	Extent of coronary collaterals			Angiogram	Flow study
			Transpleural	Intercoronary	Retrocardiac		
	mos.						
Group B							
Polyethylene wrapping of left main coronary artery							
341	4	+++	+++	0	0		
350	4¾	++++	+++	0	0	*	
351	5¾	++++	0	0	0	*	
336	6	++++	++++	+	+++		
352	6	+++	+++	0	+++		
335	7	++++	++++	+	++	*	
Group C							
Polyethylene wrapping of both main coronary arteries							
353	6	++	+++	0	+	*	*
355	6	+++	++	0	0		*

Key (pulmonary collaterals):
+ = Slight expansion of bronchial arterial trunks at source.
++ = Moderate expansion to proximal bronchi only.
+++ = Filling to distal bronchi.
++++ = Filling of fine vessels about distal bronchi and retrograde injection of pulmonary arteries.
* = Special study named above was made.

In some specimens, filling of a plexus of tortuous small arterial vessels in the lung occurred easily from the coronary arteries, and these vessels decreased in caliber with increasing distance from the heart, while it was difficult or impossible to inject the coronary arteries from the bronchial plexuses. This suggested that in these instances the coronary arteries were acting as a source of collateral circulation to the lung. In almost all specimens, however, each system could easily be filled with plastic from the other.

In many casts the pulmonary arteries also were filled with plastic, either with the red material that had been injected into the coronary arteries and that had entered the lung via the transpleural and bronchial collaterals or, more commonly, with the black material that had been introduced into the bronchial system more directly from the

aorta (Fig. 3). This maintained patency of the pulmonary artery after its ligature at the hilum, and the expanded connections with the bronchial arteries that make possible a retrograde injection from the

TABLE III

Development of Collaterals to Myocardium after Ligature of Left Pulmonary Artery and Cardiopneumonopexy

Group D_1

Unsuccessful attempt at interruption of left anterior descending coronary artery

Dog no.	Interval: stage I-stage II	Interval: cardio-pneumonopexy to sacrifice	Extent of pulmonary collaterals	Extent of coronary collaterals			Angiogram	Flow study
				Transpleural	Intercoronary	Retrocardiac		
	wks.	*mos.*						
334	8	3¼	++	++	+	0	*	
325	4¼	6	++++	++++	+	0	*	
329	4	7	+++	++++	+	+++		
315	8	9¼	+++	++++	0	0	*	
316	7	9¼	++++	++++	0	++++		
324	6	11½	++++	+++	0	+++	*	*
320	6	12¼	++++	++++	0	+	*	*

Group D_2

Successful interruption of left anterior descending coronary artery

314	12	4	++	++	+++	+		
321	3¾	11¾	+++	++	++++	0	*	*

Key (pulmonary collaterals):

\+ = Slight expansion of bronchial arterial trunks at source.

++ = Moderate expansion to proximal bronchi only.

+++ = Filling to distal bronchi.

++++ = Filling of fine vessels about distal bronchi and retrograde injection of pulmonary arteries.

* = Special study named above was made.

periphery have long been known and have been demonstrated previously by the plastic casting method in this laboratory.[48] Lezius[13] also noted connections between transpleural collaterals and the pulmonary arteries.

The Retrocardiac Collaterals

In approximately 50 per cent of the animals there was an expansion of vessels that can best be designated as retrocardiac. The term extracardiac has been employed for some of these vessels, but this would hardly distinguish them from the transpleural collaterals that have

been described. The constancy of these arteries would suggest that they represent expanded pre-existing vessels.

The most frequently observed and largest of these retrocardiac collaterals was in continuity with the anterior left atrial artery (Figs. 4 to 6). The latter, as carefully described by Meek and his co-workers,[86] branches from the left circumflex coronary artery within 5 mm. of the origin of the latter. Its course is then over the base of the auricular appendage. The diameter of this vessel in its expanded state sometimes equalled that of the distal circumflex coronary artery (Fig. 6). In this state it continued into the hilum along the superior and posterior surfaces of the left superior pulmonary vein and became continuous with the enlarged bronchial vessels about the bronchi, or with the subcarinal arterial plexus. Such collaterals were not injected in any of five control casts.

Distal retrocardiac arteries also were present in some instances. These originated on the posterior aspect of the heart from the circumflex, close to the point of origin of the posterior descending coronary artery. The position and course of these vessels indicate that they represent the left posterior atrial branch in an expanded state. Again multiple connections with the bronchial arteries were present (Fig.7). The tortuous collaterals ascended on the posterior surface of the left atrium and inferior pulmonary vein and then passed between the major veins of the left side to join the bronchial arterial plexuses.

TABLE IV

Sources of Bronchial Collateral Vessels in 23 Dogs

Principal vessel	All vessels	Number of dogs
A^1	A^1	5
	$A^1 + A^2$	3
	$A^1 + A^3 + A^2L$	1
	$A^1 + A^4$	1
A^2	A^2	4
	$A^2 + A^3 + A^2L$	1
A^3	A^3	2
	$A^3 + A^1 + A^4$	1
	$A^3 + A^2$	2
A^4	A^4	2
Pericardiophrenic branch of innominate		1

Key: A = Aortic intercostal artery at level indicated by superior number.
L = Branch derived from left intercostal artery.

The Intercoronary Collaterals

Although anastomoses among the coronary arteries could not be demonstrated by the vinylite method in five control casts, intercoronary collaterals exceeding a diameter of 50 μ were found in 7 of the 25 dogs of the experimental series as early as 15 weeks after cardiopneumonopexy, and they reached a large size only in those 2 animals

in which the interruption of the major coronary artery had been complete and permanent. In one of these (Fig. 8) the patency of the distal segment of the ligated left anterior descending artery was maintained, in part, by a tortuous collateral vessel that connected it with the posterior branch of the circumflex, without loss of caliber as it continued over the apex of the heart; there were additional transpleural connections. Although the vessel was patent beyond the point of interruption, an infarct had occurred. In the other animal (Fig. 9), a coarse network of collaterals connected branches of the right coronary artery and other branches of the left with the distal ligated ramus of the left anterior descending artery. Transpleural and deep myocardial collaterals also were present. In the other animals, intercoronary collaterals were less direct and resulted from a participation in the transpleural plexus of components of more than one of the major coronary arteries.

Only in a single animal in which the left anterior descending artery had been successfully and permanently ligated was there evidence of the expansion of precapillary vessels that bridged the gap created by the interruption of the artery. This plexus lay deep within the myocardium and represents a special form of intercoronary collateral (Fig. 9).

As a consequence of the various types of collaterals, red plastic injected into the coronary arteries sometimes escaped freely into the descending thoracic aorta, and was seen within the bronchial arteries mingled with black after the cast was made (Fig. 10).

Discussion

Necessity for an Adequate Injection Method in the Study of Collateral Circulation to the Heart

It is clear that an injection method is indispensable to establish adequately the possible channels of blood flow. The vessels are for the most part too small for dissection, and even the larger may be buried within adhesions firmer than the tissue of which they are composed. Injection should be with the heart *in situ* and should be carried out not only from the ostia of the coronary vessels, but also from the entire thoracic aorta beginning in its ascending portion beyond the sinuses of Valsalva. Only in this way will the retrocardiac vessels, in particular, not be missed. This procedure is especially important after interruption of any of the coronary arteries. Entirely unsupported, in the absence of such an injection procedure, are implications regarding Thebesian,[3,47] pericardial,[1] or septal vessels as representing the sole

vascular support of the myocardium. Coronary arteries usually remain patent beyond a point of obstruction as has again been demonstrated in the present experiments (Figs. 8 and 9).

Injection also is necessary for proof that a "ligated" coronary artery has in fact been permanently interrupted, as indicated by the present experience. It is certainly best to place double ligatures and to cut the vessels between them, and to obtain confirmation by injection at the end of the experiment. The injection, moreover, will demonstrate whatever vessels may have bridged the gap.

Adequate controls are necessary in any injection procedure. It may be assumed that the capillaries of all organs are connected, devious as the connections may be. Therefore, the viscosity of the injection medium should be such as to prevent penetration beyond vessels of arteriolar size. Doubtless mechanical factors account for differences in statements regarding the presence of "normally" occurring anastomoses of coronaries.[48-52] Technical factors are especially difficult to control when non-homogeneous media are used, when temperature affects the viscosity of the medium (as in the case of gelatin or agar suspensions), or when contact with moisture causes hardening or precipitation (as with the vinylite of the present experiment). There are also, in the case of the plastic polymers, unpredictable variations in the stock material. Consequently, the same material prepared under identical conditions should be employed in a standardized fashion for control and experimental animals.

Necessity for Correlated Functional and Anatomical Evidence

An anatomical connection between two vascular beds, such as that of the coronary and bronchial arteries in the present system of collaterals, is simply a bridge, the presence of which gives no information as to the volume or even direction of traffic. This principle has been emphasized by Burchell.[20] To obtain a full understanding, the anatomical observations must be supplemented by functional studies, as has been done in the course of the present experiments. Thus it has been demonstrated that even without the presumed "demand" created by interruption of the pre-existing vascular supply of the myocardium, some blood from the collateral vessels reaches the coronary sinus within the observation period of 30 seconds. It is possible that this flow is phasic or even reversible during a single cardiac cycle. For this reason the collateral circulation should be studied with the heart as close as possible to a state of nature, that is without the introduction of artificial pumps or heads of pressure, as has been attempted.[22]

Whatever the direction of flow when the coronary bed is intact, the existence of a large vascular connection with the collateral system will permit blood from the latter to supply a capillary bed beyond a point of occlusion and anastomosis. A change in direction of flow to a distal distribution where the pressure has become diminished will occur in the cardiovascular system, as it does in any hydraulic system. The intercostal vessels that bring blood *toward* the aorta beyond a coarctation furnish an example in point. In the coronary arterial bed, a functionally "end-arterial" system,[52] it is important that the anastomoses be available at the moment of occlusion. The cardiopneumonopexy after induction of the expanded bronchial arterial bed meets this requirement, since large precapillary anastomoses have been demonstrated in the absence of "need"; that is with a coronary bed the integrity of which has not been impaired.

The anastomoses that have been demonstrated are definitely of precapillary size, at times exceeding 1 mm. in diameter of lumen, and are directly connected with coronary arteries and arterioles. This implies that blood entering the coronary arterioles from the side of the anastomoses must pursue the same course to capillaries of the myocardium as blood reaching the arterioles in the intact animal. Never has a direct connection exceeding capillary size to the branches of the coronary sinus or other cardiac veins been evident in the present material. Such oxygenated collateral blood should therefore be useful to the cardiac muscle fibers in the same way as oxygenated blood arriving normally in the coronary arteries from the sinuses of Valsalva.

Possible Stimuli to the Development of Various Types of Collaterals

Retrocardiac Collaterals. Perhaps the most interesting of the collateral vessels are the retrocardiac vessels. For the most part these are in continuity with identifiable atrial branches, as has been described in detail previously. It is highly probable, therefore, that they represent expanded pre-existing vessels that can be demonstrated to connect with bronchial and other mediastinal vessels in normal animals by the use of injection media of low viscosity. However, the vinylite, as used in 5 control dogs of the present experiments, will not demonstrate vessels of such small size in normal animals. With the enormous expansion of the proximal bronchial vessels, their atrial extensions expand *pari passu,* as is true in any arterial bed. Recent observations in this laboratory, which will be reported in detail, indicate that this enlargement of the retrocardiac plexus is independent of the establish-

ment of a cardiopneumonopexy, and is associated simply with the ligature of the pulmonary arteries. Some mechanisms concerned in this association have been discussed elsewhere.[53]

In man there exist not only retrocardiac vessels that follow the course of the pulmonary veins as in the dog, but also mediastinal arterial branches derived from the descending thoracic aorta that enter the interatrial septum from the rear (Fig. 11). Their rôle as possible collateral vessels remains to be explored further. They have escaped extensive study in the past since few human hearts have been injected *in situ* from the descending thoracic aorta as well as from the coronary ostia.

Transpleural Collaterals. After ligature of the pulmonary artery the lung appears to exhibit a blood-thirst that is partly met not only by expansion of the bronchial arteries, but also by the rapid growth of arterial vessels within adhesions. Thus, in the present experiments after cardiopneumonopexy, not only the intercostal vessels but also the coronary arteries come to be connected with the bronchial arteries. It is probable that the blood at first goes into the lung, at least during a part of the cardiac cycle. The direction of flow is determined by relative pressures in the peripheral capillary beds, and may at any time be reversed as determined by alterations in these pressures.

The broad surface of attachment of lung to heart achieved in the present operation might be considered *a priori* desirable as a source of collaterals, since any of the coronary arteries in human disease might be especially involved in sclerosis or some other occlusive process. Furthermore, the "bridging" action of the transpleural collaterals serves to join adjacent coronary vessels, as well as to establish connections with the bronchial arterial system.

Adhesions as Such as a Source of Collateral Blood Flow. It may be questioned whether the obliterative pericarditis and pleuritis are not in themselves sufficient to supply significant collateral circulation to the heart. Such questions can be resolved only by actual observations and measurements of flow. In dogs with talc pericarditis, studied by procedures similar to those employed in the present experiments, the transpericardial and retrocardiac collaterals were smaller, the immensely expanded bronchial arterial bed within the lung was, of course, lacking, and the observed collateral flow into the coronary sinus was much less.

The Venous Side of the Collateral Circulation. It is noteworthy that precapillary connections with veins were not found, despite extensive anastomoses of the transpleural arterial collaterals. Conversely, it has

been observed that when the pulmonary veins are interrupted only a venous collateral circulation is developed.[54] When both the pulmonary arteries and veins are ligated, both types of collaterals are produced, but systemic arteries join only the pulmonary arteries, and systemic veins invariably connect directly with pulmonary veins, even when the collaterals are newly formed, as they must be within adhesions that obliterate former serous cavities.[55] The factors responsible for these orderly events are not known.

Relation to Human Pathology

In considering what possible bearing these experiments might have on the therapy of coronary insufficiency in man, it must be stressed that different species need not react necessarily in the same way to the same surgical procedure. The capacity of the bronchial vessels to increase has, however, been demonstrated abundantly in man.[53] It would be undesirable in man to ligate the pulmonary arteries to an entire lung. The lingula in man is comprised of a mass of tissue sufficiently large to furnish a considerable collateral blood supply after the lingular artery, or arteries, have been ligated. These vessels are easily accessible surgically. Such an operation would not be applicable to patients with congestive heart failure since in them infarction of the lung is to be expected, in contrast with the normal condition.

Cardiopneumonopexy suggests itself in the treatment of transposition of the great vessels. Here the coronary arteries derive blood of extremely low oxygen saturation from the base of the transposed aorta. Since in more than 50 per cent of patients with this congenital malformation, the pressure in the pulmonary artery actually exceeds that in the aorta, and since the transposed pulmonary artery contains blood that is highly saturated with oxygen, the indications for simple cardiopneumonopexy are strong. The pulmonary artery would, of course, not be ligated on the side of the cardiopneumonopexy unless a surgical retransposition could be performed successfully, for then it is hardly conceivable that the coronary arteries could be successfully carried over with the root of the aorta.

Differences between human and canine hearts have been emphasized, especially the dominance in the latter of the left coronary artery, the existence of one major septal branch of the left coronary, and the presence of larger connections among the branches. The pig heart is more similar to that of man, and it may be that this species is more suitable for experiments on collateral circulation to the heart.[48,56] It should be stressed, however, that the coronary circulation of the dog is also

functionally an endarterial system. It may be worth determining whether the principles basic to the present experiments would apply to a useful degree in man.

Summary and Conclusions

A collateral circulation induced in a lung by ligature of the pulmonary artery can be applied to the coronary arteries by cardiopneumonopexy. The collaterals to the heart, as demonstrated in vinylite casts, can be classified as transpleural, retrocardiac, and intercoronary. Factors governing the development of the transpleural collaterals to the heart are incompletely understood, but are probably the same as those that determine the development of newly formed collaterals via adhesions anywhere to lung deprived of its pulmonary arterial inflow. The retrocardiac collaterals, fed by the same source, develop *pari passu* with the expansion of the bronchial arteries themselves. Large intercoronary collaterals appear only after interruption of the continuity of a major coronary artery.

The collateral circulation is usually well developed at 5 months after cardiopneumonopexy, but may occasionally be extensive within 3 months, even in the absence of "need" on the part of the myocardium. A collateral circulation is probably responsible for maintaining the patency of a coronary artery beyond a point of ligature, but does not necessarily prevent infarction when a major coronary artery is ligated within the first month after cardiopneumonopexy. Further data regarding the degree of protection to the myocardium are needed.

Controlled functional studies upon dogs have demonstrated that blood reaches the coronary circulation from these collaterals, at least during a part of the cardiac cycle, even in the absence of anoxia of the myocardium.[57]

It remains to be investigated whether cardiopneumonopexy, employing a relatively small mass of lung tissue with an expanded bronchial circulation, may be useful in the treatment of coronary insufficiency in man.

Simple cardiopneumonopexy appears to be indicated in the treatment of transposition of the great vessels, especially in those instances in which the pressure in the pulmonary artery exceeds the systemic arterial pressure.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Dog 290. Transpleural collaterals, 1¾ months after cardiopneumonopexy. A plexus of bronchial collateral vessels, one of which is designated by an arrow (T), passes across the pleura and epicardium to anastomose with branches of the left anterior descending coronary artery. The intrapulmonary extensions of these bronchial arteries are seen ramifying about the bronchi of the lingula that was used in pneumonopexy.

FIG. 2. Dog 316. Transpleural collaterals, 9¼ months after cardiopneumonopexy. Large anastomoses of transpleural collaterals, one of which is indicated by an arrow (T). These collaterals are continuous with the greatly enlarged bronchial arteries (B.A.) that spiral about the bronchi of the adjacent pulmonary parenchyma. A dense plexus of bronchial arteries is seen within the lower lobe, of which only a portion was used in the pneumonopexy. Figures 6 and 10 may be seen for other views of this cast.

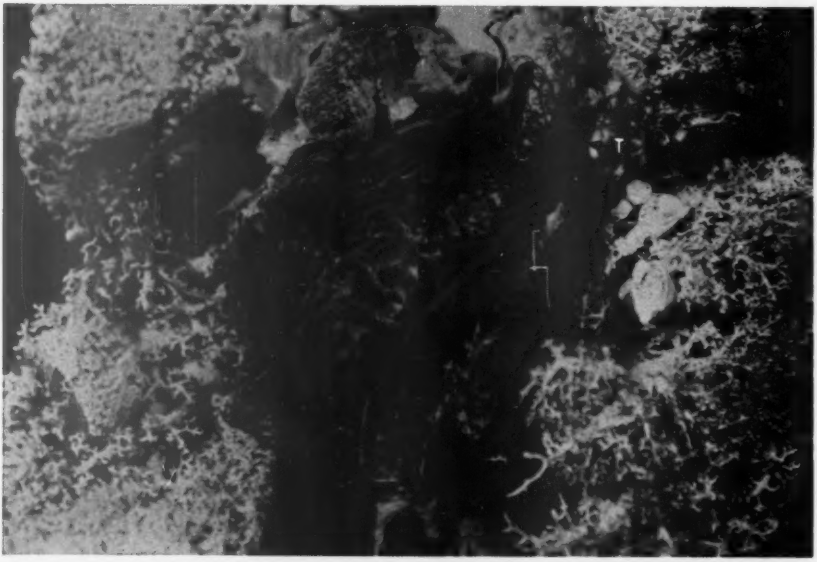

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FIG. 3. Dog 291, 2½ months after cardiopneumonopexy. The pulmonary arteries (P.A.) have been injected with red plastic by way of transpleural bronchial collaterals and the anastomoses of the bronchial vessels with distal pulmonary arterial branches. The expanded bronchial plexuses (B.A.) at the hilum also contain some red plastic received via the transpleural collaterals from the coronary arteries.

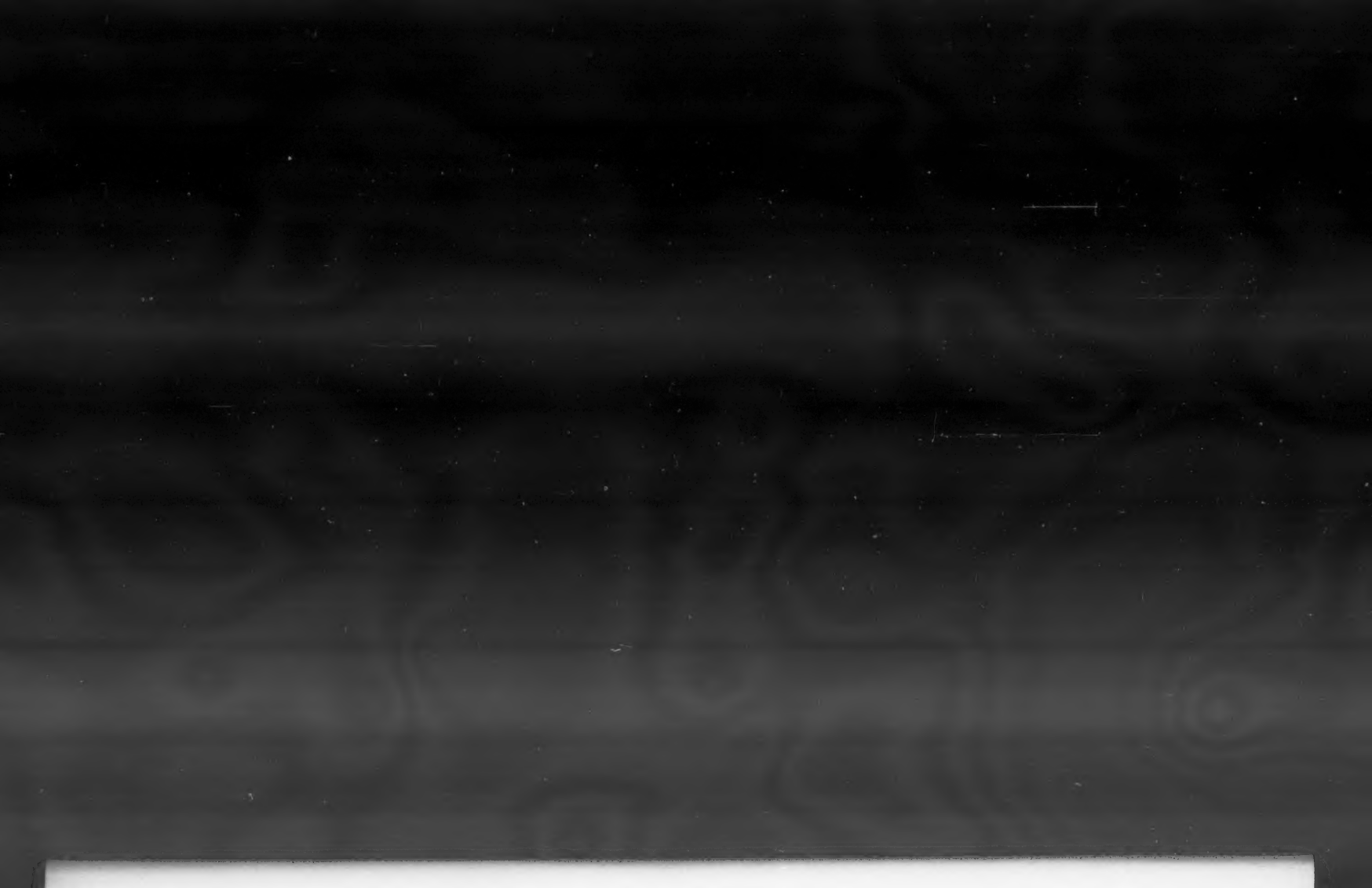

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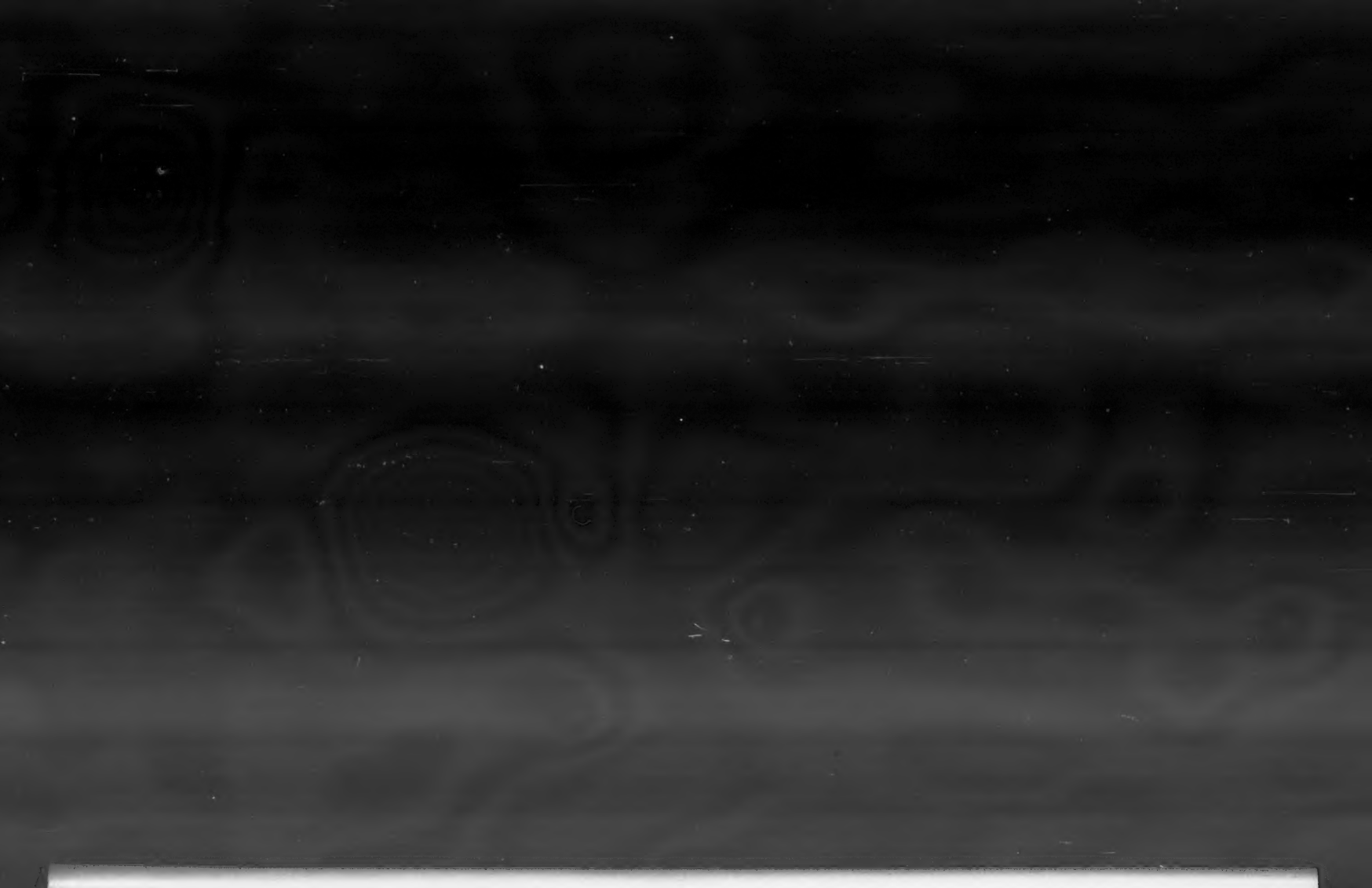
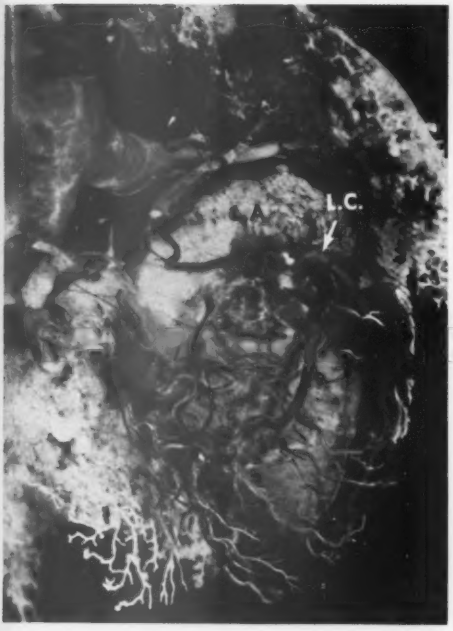
FIG. 4. Dog 352. Retrocardiac collaterals, 6 months after cardiopneumonopexy. A tortuous artery continuous with an enlarged anterior left atrial artery winds over the base of the left auricle (L.A.) and superior pulmonary vein to become confluent with bronchial vessels about an upper lobe bronchus. The anterior left atrial artery is derived from the circumflex (L.C.) within 1 mm. of the origin of the latter.

FIG. 5. Dog 292. Retrocardiac collaterals, 3 months after cardiopneumonopexy. An immense plexus of bronchial arteries is continuous with an enlarged anterior left atrial artery. The relations and labels are similar to those of Figure 4. The transected surface of the proximal aorta (Ao) is seen as a full circle with its sinuses of Valsalva, from one of which originates the trunk of the left coronary artery that immediately divides into the left anterior descending (L.A.D.) and circumflex (L.C.). The left auricle (L.A.) is also shown.

FIG. 6. Dog 316. Retrocardiac collaterals, 9¼ months after cardiopneumonopexy. In this instance the anterior left atrial artery is enlarged to equal the size of the circumflex trunk (L.C.) distally. The plexus of greatly enlarged bronchial arteries lying to the left and beneath the distal thoracic aorta (Ao) is seen to be continuous with the former. Labels as in Figure 5. Transpleural collaterals in this cast are shown in Figure 2, and the bronchial arterial connections with the aorta in Figure 10.

FIG. 7. Dog 298. Retrocardiac collaterals, 13½ months after cardiopneumonopexy. In a posterior view the circumflex is seen to be the source of an enlarged posterior left atrial artery that sweeps to the left in relation to the inferior pulmonary vein to become continuous with a plexus of bronchial arteries within the parenchyma of the left lower lobe.





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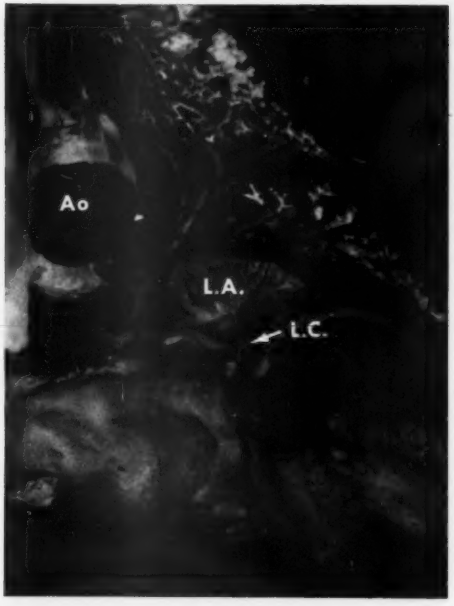

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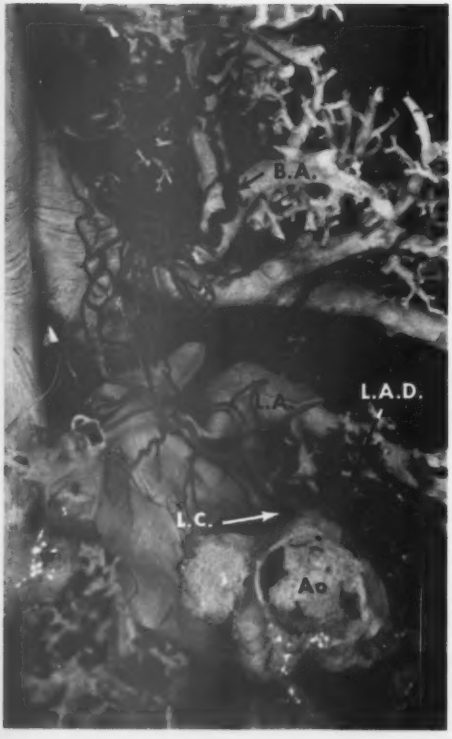

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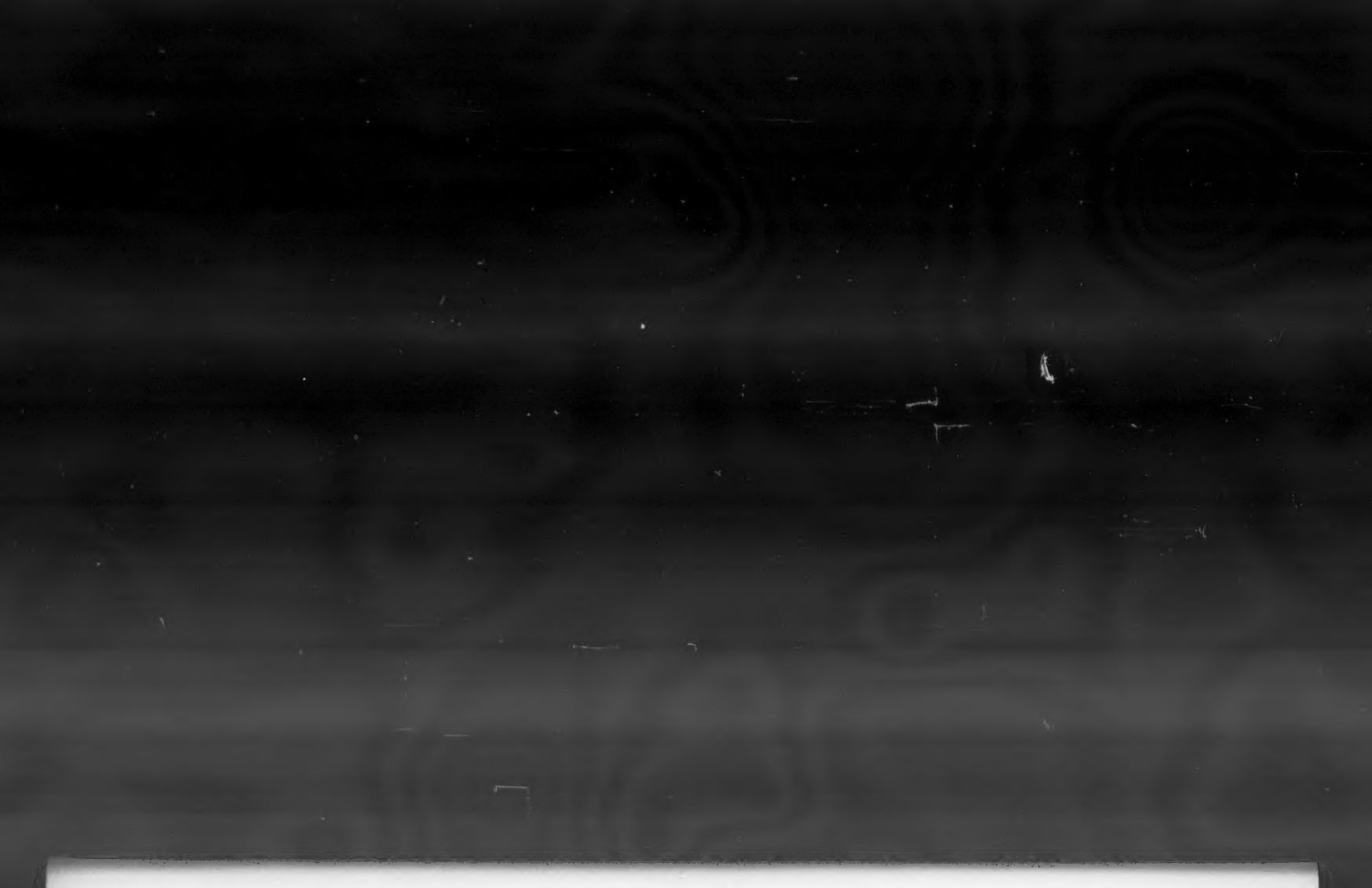
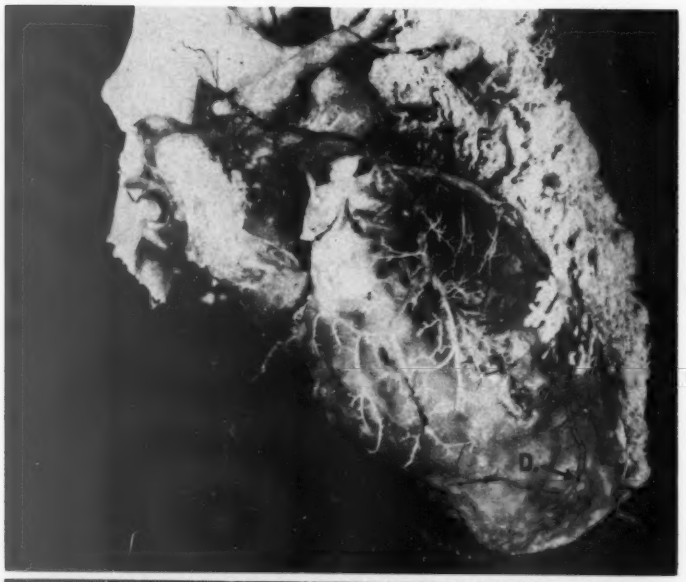
FIG. 8. Dog 321. Intercoronary collaterals. The left anterior descending coronary artery was interrupted by ligature 3¾ weeks after cardiopneumonopexy, and 10½ months prior to sacrifice. The distal segment beyond the ligature (D) remains patent, and has been injected in red from the posterior descending branch of the circumflex artery with which it is connected by a tortuous vessel that continues over the apex of the heart. A transpleural branch (T) courses from the lung to the distal segment of the anterior descending artery. The proximal segment of the left anterior descending artery (P) is injected in green from the aorta, as is the septal branch. The trunk of the circumflex artery itself was separately cannulated and injected in red. The sinuses of Valsalva are visible as prominent outpouchings of the aorta.

FIG. 9. Dog 314. Intercoronary collaterals. A large branch of the left anterior descending coronary artery was ligated 3 months after cardiopneumonopexy and 1 month prior to sacrifice. Connecting with the still patent distal limb (D) of the ligated vessel are: (1) a network of intercoronary collaterals extending from another branch (L) of the left coronary artery and from the right coronary (R); (2) a deep myocardial network joining the distal limb (D) with the proximal stump (P); (3) transpleural bronchial collaterals (T) course from their position about the bronchi to the distal limb (D).





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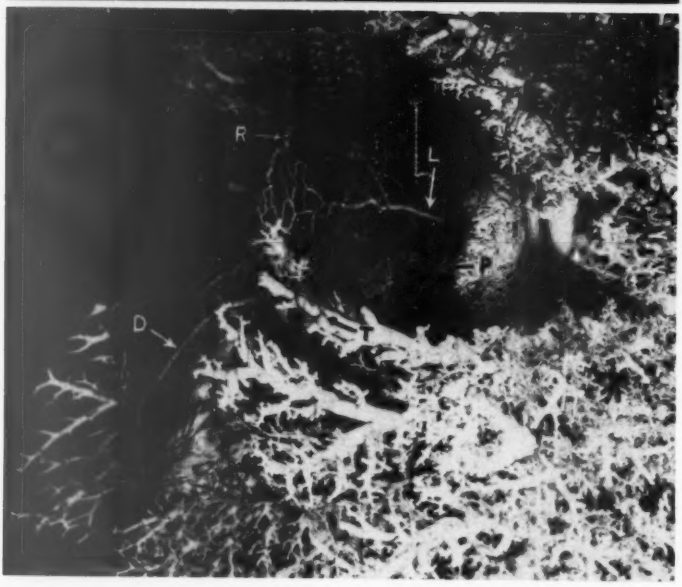

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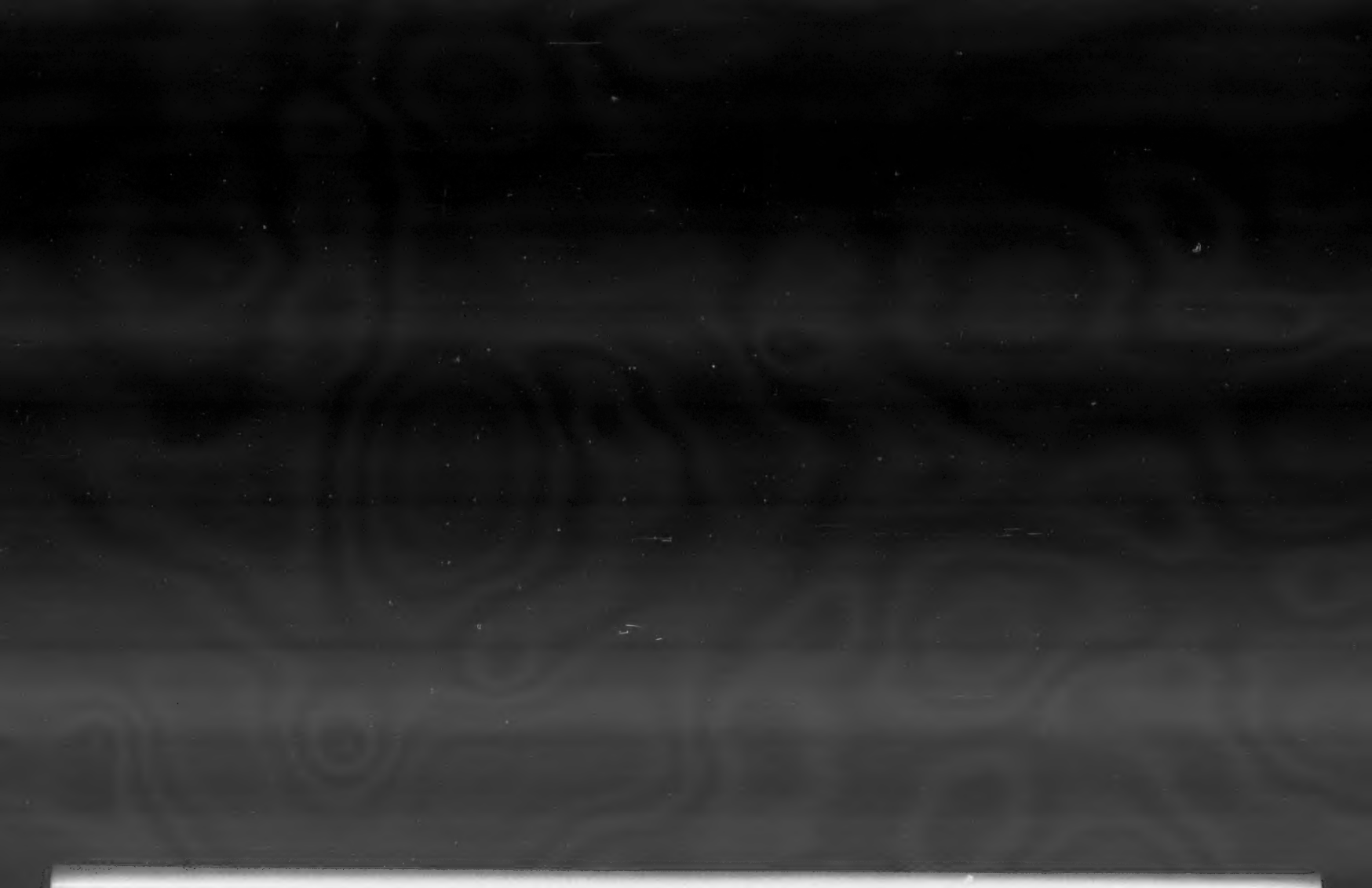
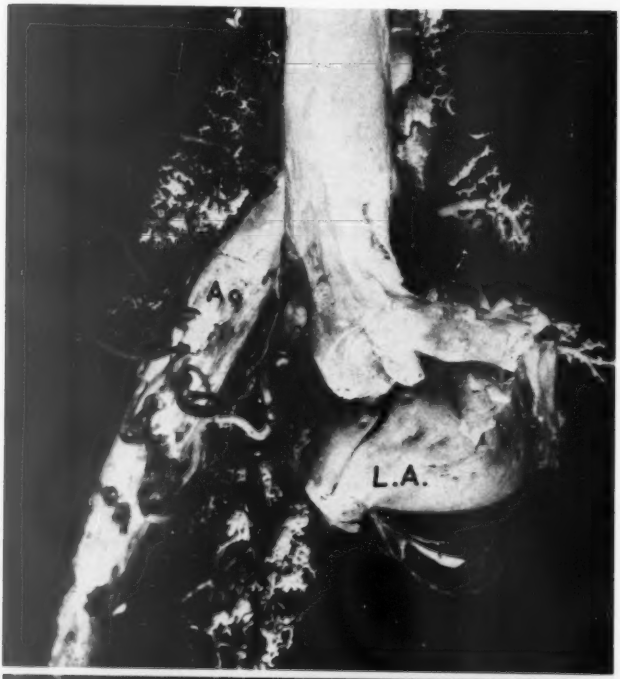
FIG. 10. Dog 316. In a posterolateral view, two huge bronchial arterial trunks are seen to contain intermingled red and black plastic. The transpleural and retrocardiac collaterals that permitted the plastic that was introduced into the coronary arteries to enter the bronchial vessels and aorta are illustrated in Figures 2 and 6, respectively. In this cast the right lung has been removed, although its main bronchi and the carina of the trachea are preserved. The aorta (Ao) is seen looping over the left main bronchus. L.A. = left atrium.

FIG. 11. Retrocardiac collaterals in man. Anterior view of a bronchovascular cast showing the aorta (Ao) in relation to the pulmonary artery (P.A.) and the left atrium (L.A.). A plexus of dark, band-like vessels lies anteriorly to the left atrium and was actually in the interatrial septum which has been digested away. These vessels were injected from the descending thoracic aorta, and are continuous with a plexus that is also the source of bronchial and esophageal arteries.





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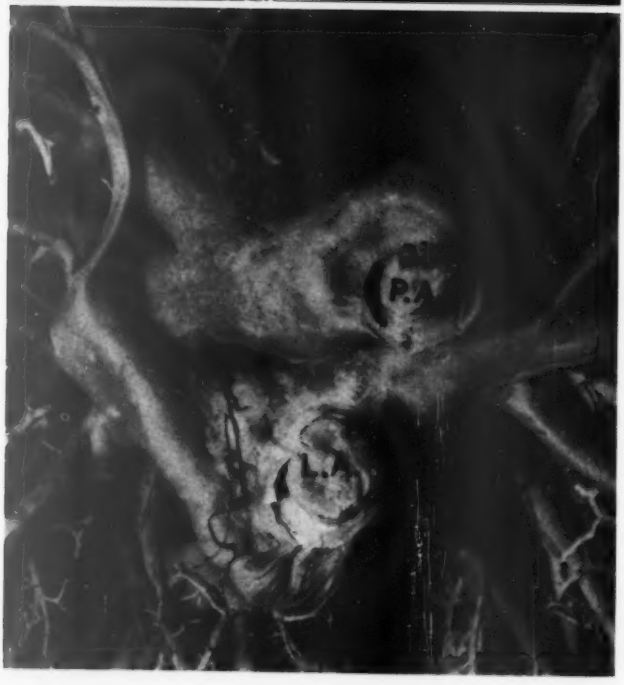

11

THYMOMA

A Review and Reclassification *

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The term thymoma with modifying nosologic designations has been applied to a host of mediastinal tumors of varying morphologic pattern. Elaborate classifications have been erected from relatively small series of cases. These classifications, in general, attempt to fit particular examples into embryologic or histogenetic categories. However, in neoplasia the thymus gland displays such freedom of expression that it conforms to none but the broadest of definitions.

The confusing literature will not be reviewed here, except to point out three general observations. First, there is agreement among some workers that the various histologic variants of the neoplastic gland are a composite of one tumor.[1,2] In accordance with this approach, the thymoma is then defined as a neoplasm in the anterior mediastinum, arising in the thymus gland and showing various mixtures of cells; it is usually benign in behavior, often encapsulated, and, in some cases, occurs in patients with myasthenia gravis. Second, it is now generally agreed that tumors such as malignant lymphomas, although sometimes originating in the lymphoid tissue of the thymus, should not be classified under the specific term thymoma. Third, until more is known about the potentialities of the thymus gland, it is best to withhold the specific designation from neoplasms of dubious origin when the only definite support for the diagnosis may be the mediastinal location of the tumor.

During the years 1950–52, as criteria for the diagnosis of certain mediastinal tumors in the Chest Tumor Registry were under revision, 50 cases from the files of the Armed Forces Institute of Pathology, diagnosed as thymoma, were studied. There were no examples which could be called thymic carcinoma; 8 of the cases classified under this designation were found to be neoplasms resembling seminoma or dysgerminoma. Five cases called thymoma were excluded because they represented reactive changes in groups of anterior mediastinal lymph nodes with alterations in the follicles which simulated Hassall's corpuscles. Ten cases were shown to be examples of Hodgkin's disease, lymphoma, and possible metastatic tumor. The remaining 27 tumors

* Presented at the Fifty-second Annual Meeting of the American Association of Pathologists and Bacteriologists, Houston, Texas, April 9, 1955.
Received for publication, August 27, 1955.

were identified as true thymomas. These were a more or less homogeneous group of localized neoplasms arising in the region of the thymus gland and composed of various mixtures of lymphocytes, pale spindle cells, cystic spaces, and large epithelial cells. In order to correlate these variations with the histologic changes of the non-neoplastic glands, thymus glands from 165 necropsy cases, ranging from premature infants to adults 70 years of age, were examined. For further information on the subject of normal growth patterns, histologic preparations of thymuses of embryos of 2 to 6 weeks were studied at the Carnegie Institution. This embryologic material was supplemented by thymus glands from fetuses aborted after gestation periods of 4 weeks to 3 months.

The purpose of this paper, based on the results of this study, is (1) to define thymoma as a single entity showing variations in morphologic pattern in relation to the presence or absence of clinical evidence of myasthenia gravis; (2) to define and differentiate the morphologic features of mediastinal seminoma, formerly called thymic carcinoma, and to define the criteria by which it is to be distinguished from true thymoma, and (3) to point out the anatomical differences between thymoma and reactive hyperplasia of mediastinal lymph nodes so that common errors in interpretation may be avoided.

In the preliminary review of the 27 neoplasms retained in this series as examples of thymoma, two consistent histologic patterns became apparent, and these were found to be related to the presence or absence of clinical evidence of myasthenia gravis. In the group with myasthenia gravis, epithelial cells were prominent. The remaining tumors showed a prominence of lymphocytes, spindle cells, or other structures resembling the stromal elements of the gland.

Thymomas Without Myasthenia Gravis

In 14 of the 27 cases, the patients had no symptoms suggestive of myasthenia gravis. The average age of the group was 50 years, and the range was from 24 to 71 years. The selection of material did not lend itself to correlation with race or sex of the patients. The clinical symptoms were those which might be anticipated from the presence of a slowly growing benign mediastinal mass. Although some tumors were removed immediately after roentgenologic diagnosis, others were observed for as long as 8 years before operation was performed, and one was an incidental finding at necropsy.

A representative case history follows:

A.F.I.P. Acc. 176626. A 28-year-old man experienced sudden onset of severe, steady pain in the right side of the chest, for which he was treated with penicillin. A

mediastinal tumor, seen on roentgenologic examination, was removed by thoracotomy. The tumor measured 4 by 5 by 7 cm., and was lobulated and encapsulated. A central cystic cavity was present in an otherwise firm tumor, which at first was considered to be a malignant lymphoma. Four years later the mass recurred and encroached upon the bronchi and vessels. Histologic preparations of a dissected portion then showed a neoplasm composed largely of pale spindle cells.

MORPHOLOGIC CHARACTERISTICS

On gross examination, the 14 tumors averaged from 8 to 10 cm. in diameter and the largest was 15 cm. All were circumscribed and more or less encapsulated. The tumors were generally soft, white or yellowish white, and sometimes contained cystic areas. The inner surface of the fibrous capsule tended to split and dip into the body of the mass, dividing the neoplasm into lobules. These fibrous septa were particularly prominent in those tumors with marked cystic alterations. The capsules were sometimes thickened by deposits of calcium and by collections of fat undergoing phagocytosis.

The histologic structure of the tumors was represented essentially by the stromal and cellular elements that are normally present in the thymus, but which, in the neoplasms, varied considerably in arrangement and quantity.

The tumors were best grouped according to the prevailing components: lymphocytes, spindle cells, and supportive structures. By so doing, it was possible to make interesting comparisons with the stages in enlargement and regression of the normal and abnormal non-neoplastic thymus glands seen incidentally at necropsy. It should be noted, however, that these stages could not be correlated chronologically with the duration or size of the tumor in a series so small.

Lymphocytic Proliferation

In thymomas of patients without myasthenia gravis and showing lymphocytic proliferation, the tumor was divided by fine cellular septa into large lobules consisting mostly of small round cells interspersed with occasional pale reticulo-endothelial cells (Fig. 1). Sometimes the septal divisions were so light that the gland seemed to be uniformly replaced by lymphocytes, and a diagnosis of lymphosarcoma was entertained. However, careful examination always showed a reticulum framework, more or less uninvaded, or unaltered, by the overflow of lymphocytes. There was little vascularity of the gland and correspondingly little fibrosis. Hassall's corpuscles were rare. When they occurred, most of them lay in a thin rim of thymic tissue, which might be located at the periphery of the tumor. In thymomas of this type, the lobular lymphoid hyperplasia was reminiscent of the appearance

of the thymus gland when it is at its largest after birth, or of the thymus gland in certain abnormal conditions such as anencephaly, hyperthyroidism, or hypoadrenalism (Fig. 2).

Spindle Cell Proliferation

In the tumors of patients without myasthenia gravis and showing spindle cell proliferation, the capsule was more dense than in those in which lymphoid cells predominated. The septa also were correspondingly prominent, dividing the lobules more definitely from one another. Small cystic spaces, which appeared to be lined by endothelial cells, lay within the fibrous septa and extended into the lobules. These were connected to the ramifying vessels and sinusoids which seemed to branch out from the septa into the lobules (Fig. 3).

The septa were rich in elongated spindle cells, extending into the periphery of the lobule with the penetrating vessels (Fig. 5). Similar changes occurred within the lobules. Whether by actual transformation of lymphocytes or by invasion of cells from the reticulum framework of the gland, the cells at the periphery of the lymphoid lobules became oval or elongated and the nuclei pale. Masses of these cells merged with the identical spindle-shaped cells in the vascular septa. The cells were uniform and did not reproduce by abnormal mitosis. They were most prominently displayed around blood vessels, but also were seen amassed in small clumps embedded in the lymphoid tissue.

Many lymphoid lobules might disappear altogether in the transformation, and in some cases the tumor seemed almost entirely replaced by the cystic, fibrous, and vascular network interlaced by the pale spindle cells peeling off from vascular spaces. Misinterpretation of the nature of the spaces lined with cells appearing to be endothelial cells, and of the pale spindle cells, has led to such confusing diagnoses as perithelioma, spindle-cell sarcoma, neurofibroma, or diffuse reticular thymic carcinoma.[3] Mediastinal paragangliomas also have been confused with thymomas showing these changes.

As in the thymoma with lymphoid proliferation, this process is reminiscent also of some of the changes in thymus glands. It is noted in the normal thymus gland that with increasing thickness of the capsule and of the septa, the lymphoid elements become less prominent. Usually, adipose tissue replaces the involuting lymphoid tissue. However, unusual regressive changes may occur in some individuals. In these, the small round cells are replaced by pale spindle cells, and the entire gland becomes atrophic and very vascular. Under these circumstances Hassall's corpuscles are rare or atypical.

These changes were seen most strikingly in a thymus gland, weighing between 2 and 3 gm., removed at necropsy from an 8-months-old child with scurvy. No lymphoid tissue remained in the atrophic cortex, which consisted of several finger-like extensions composed only of pale spindle cells threaded by vascular spaces. The medulla was restricted to the central portion of the tiny gland. The few Hassall's corpuscles remaining there were small, sometimes cystic, and associated with patent vessels (Fig. 4).

These alterations—vascularity, relative absence of Hassall's corpuscles, and replacement of lymphocytes by small spindle cells—suggest that the intermediate stage of thymomas unassociated with myasthenia gravis may be one of unusual regressive change in certain of the "stromal" and, possibly, epithelial components of the neoplasm, or of proliferation of these epithelial elements without maturation. This would explain the tumors characterized by small round cells distinguished from lymphocytes, as well as those composed of more obviously spindle forms.

Stromal, Endothelial, and Vascular Proliferation

In thymomas of patients without myasthenia gravis and showing stromal, endothelial, and vascular proliferation, the cystic areas predominated, and the solid cellular portions of the neoplasm were reduced to small islands scattered between the spaces lined with cells resembling endothelial cells (Fig. 6). The stroma might be hyaline or fibrous, and it was richly vascular. Lymphocytes or spindle cells were sometimes sprinkled along fibrous strands between the cysts. This appearance, in its extreme, suggested lymphangiomatous formations. Generally, however, these advanced changes were limited to a portion of the tumor, while the remaining lobules showed other stages such as lymphoid or spindle cell proliferation. These changes have been well described by Hubbell and Liebow,[4] who interpret the tumors as representing vascular neoplasia. However, numerous gradations among the subgroups were noted and it was apparent that even though one component might predominate, all elements participated in producing the characteristic histologic appearance of each neoplasm.

Thymomas Associated with Myasthenia Gravis

The average age of the 13 patients with thymomas associated with myasthenia gravis was 38 years. It is significant that these tumors were of relatively short duration, most of them being removed months after the first symptoms. The longest history was 7 years. In all cases

in which prostigmine was used, there was no demonstrable, consistent, histologic change in the size or appearance of the gland which could be correlated with quantity or duration of the therapy.

Although these tumors were sometimes diagnosed as "carcinoma" or "lympho-epithelioma,"[5] in no instance had metastasis occurred.

A representative case history follows:

A 41-year-old man was admitted as an emergency patient because of great difficulty in breathing and inability to sleep. Three months previously he had noticed weakness in the upper eyelids and generalized fatigability, followed by weakness of mandibular muscles and respiratory difficulty. Prostigmine produced considerable improvement. However, a relapse in symptoms occurred and by the time the patient was admitted it was necessary to reinforce breathing with a Drinker respirator. In spite of increased doses of prostigmine the patient became weaker. He was unable to raise phlegm, and gradually became less able to eat. Decline was rapid and he died 5 months after the onset of symptoms. At necropsy, a lobulated mass, 5.8 by 4 cm., was found anterior to the aortic arch in the superior mediastinum. The mass was hard and completely encapsulated. Several small cystic spaces were noted in its substance. There was no extension to surrounding structures.

MORPHOLOGIC CHARACTERISTICS

Grossly, thymomas associated with myasthenia gravis were usually circumscribed but might be lobulated. Occasionally, extensions protruded from the tumor and became separated by a pedicle from the parent mass. Usually the capsule remained intact. The tumors were soft. Unlike thymomas without associated myasthenia gravis, complex variations due to fibrous and cystic changes were infrequent, but did occur.

The most striking first impression of any microscopic field of the usual thymoma associated with myasthenia gravis was that of the loose association of lymphocytes and large watery pale cells in a succulent vascular bed. There was very little stroma and only faint reticulum to give body to the neoplasm. Thin-walled vessels coursed through all portions of the tumor in no particular arrangement (Fig. 7).

The relative proportions of lymphocytes and large epithelial-appearing cells varied from field to field and from tumor to tumor. The presence or absence of lymphocytes in this series was unrelated to symptoms in the patient. However, the large cells, when present, usually indicated that the patient had a history suggestive of myasthenia gravis. Cells of this type had a large, oval, watery nucleus, approximately 25 to 30 μ in diameter, and one or two very prominent nucleoli. The cytoplasm, when visible, might be clear or granular and the cell outlines oval, columnar, or polyhedral. In most examples, these cells were loosely mixed with lymphocytes but also in many areas occurred in small clumps throughout the tumor. In those examples in which lymphocytes were scarce, there was a tendency toward

greater organization of the type-cells. In large numbers they became confluent, and then almost syncytial in arrangement (Fig. 8). Usually, whether in small or large clumps, these cells were seen in intimate relationship with blood vessels, and when they lined up in cords around vascular spaces they tended to assume a more columnar shape (Fig. 9). With the periodic acid-Schiff stain, there may be noted finely dispersed red granules in the cytoplasm of the cells lining the vessels.[6]

In some areas the cytoplasm of the large cells became scant. The cells then assumed a more oval shape and might fall into concentric whorls resembling Hassall's corpuscles (Fig. 10). As more examples accrue, some of the unusual variations of tumors of this group may be found to be due to such changes in shape and in organization of the type-cell.

The relationship with blood vessels seems to be of particular significance. The innermost structure is generally a thin-walled capillary partly surrounded by a large crescentic lymphatic. In most examples the lymph vessel contained the usual circulating lymphocytes and monocytes, but in some the lumen was plugged with foamy macrophages which were noted also among the cells of the surrounding tumor. In some of the neoplasms the lymph vessel was no longer visible except as a faintly eosinophilic amorphous band in the customary relationship to the still patent capillary.

The fact that Hassall's corpuscles may be formed by epithelial cells suggests that these cells, not the lymphocytes, should be termed thymocytes. Further, their morphologic appearance when in relationship to blood vessels indicates the possibility of a period in the growth of the thymus during which an endocrine function may be maintained by the developing gland. In the thymuses removed from fetuses at necropsy there were scattered foam cells, a prominent medulla, and a relationship between Hassall's corpuscles and blood vessels; however, no cells similar to the type-cell of the thymoma in myasthenia gravis could be seen in these fetal glands or in those dissected from early embryos. More extensive embryologic studies will have to be undertaken before a direct relationship can be demonstrated, if any exists.

Comparison of Thymomas With and Without Associated Symptoms of Myasthenia Gravis

The symptoms produced by a slowly growing mass in the anterior mediastinum are common to thymomas with and without associated myasthenia gravis. In this series, the thymomas not associated with myasthenia occurred in an older age group. This may indicate that in the older age group they represent a phase of regression of

active tumors. However, in neither group was clinical correlation of the histologic appearance of the gland with prostigmine therapy, or with the progress of symptoms in the patient, adequate to permit conclusions on this point.

Because the thymoma in a patient without myasthenia gravis represents neoplasia predominantly of the stromal or non-functioning elements of the gland, this group contains more examples in which cyst formation and fibrosis are prominent. However, there is no clear-cut characteristic generally useful in gross differentiation.

The histologic differentiation of the two types depends upon basic understanding of the organization of each. The tumor associated with endocrine symptoms is recognized by the peculiar arrangement of large pale cells around thin-walled vessels and the loose association of these cells with lymphocytes in the "pulp" of the mass. This pattern and these cells in this form are not noted in thymomas in which one or another of the non-functioning elements separately enlarge and dominate the neoplasm. Instead, one may note replacement of the tumor by lymphocytes or by small pale spindle cells. Or, a more complex arrangement may result from the appearance of heavy fibrous septa which penetrate the bulk of the mass and divide it into lobules. It is probable that as time elapses the vascularity increases and formations resembling endothelium become more evident while the cellularity diminishes.

"Thymic Carcinoma" and Seminomatous Tumors of the Mediastinum

In 1946, Friedman and Moore,[7] in an extensive review of neoplasms of the testis, suggested an interrelationship between teratocarcinoma and other testicular tumors such as embryonal carcinoma, choriocarcinoma, and seminoma. Although this relationship is challenged by other workers,[8] the histologic criteria of choriocarcinoma and seminoma or dysgerminoma are more or less well defined, and their various sites of origin, in common with teratomas, are recognized. Since teratocarcinomas are known to arise in the mediastinum, it is not surprising to find reports of primary mediastinal choriocarcinoma,[9,10] or even the less well defined embryonal carcinoma. In view of these findings and the increasing numbers of reports of mediastinal teratoma, it is even more noteworthy that the only recorded examples of seminoma or dysgerminoma of the anterior mediastinum are the two reported by Friedman[11] as originating in the thymus.

In the present series of 50 anterior mediastinal tumors, those thymomas classified as benign and those called thymic carcinoma were

grouped separately. It was then readily appreciated that the histologic pattern of the two differed radically and that the tumors called thymic carcinoma were similar to seminoma or dysgerminoma. These conformed to some reported cases of thymic carcinoma of the granulomatous type.[12] Eight of the 50 cases, whether or not they seemed to originate in the thymus, were consequently reclassified as mediastinal seminoma and, together with three additional contributions from other sources, were studied as a group.

In this series of 11 examples of mediastinal seminoma, 9 of the patients were males with an average age of 24 years. Although the mass in 3 patients was discovered during routine x-ray surveys, in the majority roentgenograms were made because of pain or other symptoms referable to a slowly invasive mediastinal tumor. In one patient an Aschheim-Zondek test was positive, but there were no signs in the other patients by which the tumor could be distinguished clinically. None of them had weakness or paralysis suggestive of myasthenia gravis. X-ray therapy, when employed in 2 patients, caused remarkable shrinkage of the tumor. In some instances, however, both surgical and x-ray treatment was followed by recurrence or metastasis after a period of months or years.

There was widespread extrathoracic metastasis in one case studied at necropsy, but, in another, death was, more typically, the result of local invasion of the lungs and mediastinal structures. This tendency to local extension was demonstrated in two other individuals in whom the tumor was first diagnosed clinically by biopsy of cervical nodes. In one of these, the spinal cord became involved almost 2 years after the metastasis was discovered in the nodes of the neck.

A representative clinical history follows:

A.F.I.P. Acc. 164308. A 20-year-old white man was admitted to the hospital because a mediastinal tumor had been observed on x-ray examination during his separation from the Army. There were no previous symptoms other than asthma and frequent colds. The only contributory physical findings were directly related to the presence of the mass. Exploratory thoracotomy showed the tumor to be so attached to the inferior vena cava and innominate veins that it was considered inoperable. A small piece was removed for histologic examination. Postoperative x-ray therapy resulted in rapid shrinkage of the mass and during the subsequent 3 years the patient remained well except for cough and mild dyspnea. During that time roentgenograms showed no recurrence.

MORPHOLOGIC CHARACTERISTICS

Grossly, primary mediastinal seminomas were usually circumscribed and lobulated, but were not necessarily encapsulated. They might attain a size of 18 to 20 cm. or more. At operation they were described as spongy, soft, and grayish yellow. The stroma was not especially

dense. The tumors often were found to be attached to the surrounding great vessels and for this reason might be inoperable.

Microscopically, the tumor was honeycombed by thin fibrous and reticulum strands dividing it into acini in which clustered the neoplastic cells (Fig. 11). Lymphocytes were scattered throughout the vascular fibrous stroma which was boldly outlined by reticulum stains. This basic pattern was seen uniformly throughout the neoplasm.

The type-cell of the primary tumor was distinctive. It was large and irregularly rounded. The nucleus had coarsely clumped chromatin and was fairly uniform in size and in staining quality. Sometimes the cells formed almost a syncytium within the acini and in those instances their cytoplasm was clear and slightly swollen. In other tumors, they were seen to cluster loosely, and the cytoplasm then formed a dark band outlining the coarse nucleus. When this occurred, the lymphocytic infiltration in the septa was more prominent and varying degrees of reticulo-endothelial reaction, not correlated with x-ray therapy, might be noted (Fig. 12).

This reticulo-endothelial reaction seemed to originate in the stroma of the tumor. It consisted chiefly of a local proliferation of the histiocytes abutting on the acini. The cells were easily distinguished from the neoplastic cells by their pale nuclei and abundant eosinophilic cytoplasm. In the more extreme reactions many lymphocytes and plasma cells were seen; giant cells were noted (Fig. 12) and even birefringent particles could be demonstrated in their cytoplasm. Epithelioid tubercles might be formed. If the reaction was of sufficient proportion, the tumor cells might be overlooked (Fig. 13) and, depending on the types of reactive cells present, a diagnosis of Hodgkin's disease or sarcoidosis might result.

Definite inclusions indicative of a relation to teratoid tumors were noted occasionally in this series (Fig. 14). In one, several small foci of cells resembling trophoblastic elements were demonstrated; in another, glands; and in a third, nervous tissue and cartilage. Transitions to embryonal carcinoma were reported in a case not included with this series.[11]

Poorly differentiated mediastinal seminomas may be expected to occur and these may be distinguished only with great difficulty from some teratocarcinomas. In one example, the uniform large round cells composing the biopsy specimen strongly suggested a tumor of this general category but the diagnosis was confirmed only when trophoblastic structures were noted. In other cases, the first biopsy of the

metastasis or of the primary tumor was histologically characteristic of seminoma, but subsequent biopsies or necropsy preparations showed such poor differentiation that the tumor could not have been classified without reference to the previous material.

Comparison of Mediastinal Seminomas and Thymomas

In summary, the justification for reclassifying these neoplasms as mediastinal seminoma, rather than as thymic carcinoma, is based on clinical and anatomical similarity to testicular seminomas and ovarian dysgerminomas, as well as their dissimilarity to clearly recognizable thymomas. The mediastinal seminoma is relatively radiosensitive, tends to occur in younger age groups, and to extend locally. Like the testicular seminoma, it also may contain teratoid, trophoblastic, or other embryonal structures which are never seen in thymomas. A study of the embryos at the Carnegie Institution and of numerous glands removed from fetuses failed to reveal any embryologic or histologic connection between the thymus and this tumor.

Contrasted to the true thymoma, which comes to clinical notice because of a routine roentgenogram of the chest or because of myasthenic symptoms, the mediastinal seminoma is detected clinically because of its invasive properties, either through local extension or through metastasis to cervical and axillary lymph nodes. While the thymoma remains encapsulated, or at least circumscribed, except in unusual cases, the mediastinal seminoma in its gross appearance is much more irregular in its shape, extension, and size. Histologically, other striking differences are seen. The thymoma occurring in patients without myasthenia gravis, regardless of its classification, is divided into lobules by connective tissue septa of varying vascularity. The lobules are composed of indiscriminate mixtures of cells which are normally found in the thymus, arranged in no rigidly characteristic pattern. In contrast, the mediastinal seminoma has no large septal divisions. It is uniform throughout. It is basically arranged in acini separated by fine connective tissue septa. There are no sinusoids and no lymphoid follicles; there is no evidence of attempts at formation of Hassall's corpuscles, and none of the histologic features normally seen in the thymus, which often carry over into benign thymomas. Granulomatous reactions may be an intrinsic part of the seminoma (Figs. 12 and 13), but are rarely seen in the tumors defined here as thymomas, except when associated with fats. Lastly, those thymomas in patients with myasthenic symptoms are characterized by a type-cell which also contrasts with that of

the seminoma. In the thymoma, the type-cell has a large watery nucleus, prominent nucleolus, and ill defined cytoplasm. In the seminoma, the neoplastic cell is recognized by its round, dark nucleus with coarsely clumped chromatin, surrounded by a thin rim of sometimes granular cytoplasm. It should be emphasized that while these differential features are generally applicable, there are still some puzzling exceptions provocative of further study. Some tumors classifiable in this group arise in the thymus gland, and are involved by such a marked granulomatous reaction that the nature of the type-cell is obscured. None of these exceptional examples was associated with myasthenic symptoms in the patient, and it is hoped that study of a larger series will clarify their relationship, either to thymogenic tumors or to the teratoid group.

Localized Hyperplasia of Mediastinal Lymph Nodes

In this series, there were 5 cases in which the revised diagnosis of "reactive lymphoid hyperplasia" was made. Since the histologic appearance of the lesion was similar to that illustrated in other series of thymomas,[3,12] these cases are reviewed here for purposes of clarification. Numerous other examples may be gathered by screening a larger sample, but because of the similarity of all only a summary description is required.

The extreme importance of the proper diagnosis of this lesion lies in its differentiation from thymoma and prevention of unnecessary surgical and psychologic trauma to the patient.

The 5 cases selected for study concerned patients whose ages ranged from 22 to 45 years, and averaged 25 years. None of the lesions produced symptoms and all were discovered on routine fluoroscopy or roentgenologic examination. In one patient, the mass remained stationary for 6 years and was excised only after a cervical node became enlarged. The node and the mediastinal mass showed a similar reaction of lymphoid hyperplasia, and enlargement and hyaline alteration of the germinal centers.

A representative case history follows:

A.F.I.P. Acc. 523088. A 22-year-old man was operated upon because of an anterior mediastinal mass discovered by routine roentgenologic examination. The mass was located in the superior mediastinum, and was loosely adherent to the phrenic and vagus nerves at the arch of the aorta. The gross specimen weighed 67 gm. and was 8 by 5 by 3.5 cm. in size. It was roughly nodular, with shaggy adhesions on some portions of the encapsulated surface. The cut surface revealed fat caught between the nodules which were a contrasting gray. On histologic examination, the mass was believed to represent a thymoma. Microscopic examination of portions of the pleura and pericardium revealed mild diffuse inflammation of these structures.

MORPHOLOGIC CHARACTERISTICS

The tumors from the cases of "reactive lymphoid hyperplasia" showed no gross distinguishing characteristics. They were generally encapsulated, and attained a size of 15 cm. or more. It is noteworthy that they tended to vary in location and were described as comprising several nodules clumped together. The cut surfaces were usually grayish with little apparent stromal pattern.

In this group and in other similar examples, the prominent histologic features were the centers of the lymphoid follicle. These might be larger and more numerous than usual (Fig. 15). At first the hyaline alteration and the whorled arrangement of the hyalin might suggest thymus gland to the observer who knew that the mass was removed from the anterior mediastinum. The hyaline "rings" might completely transform the germinal center, or pale cells might still be visible. The pulp of the gland usually was unaltered, although at times there was a more pleomorphic response consisting of plasma cells, eosinophils, histiocytes, and even giant cells. Sinusoids also were frequently obscured by an obliterative reaction which was marked by hyaline thickening of the sinusoidal walls (Fig. 16). Pale slender reticulo-endothelial cells, however, might still indicate the course of the sinusoids which reticulin stains demonstrated plainly. Associated with these changes there was thickening of the nodal capsule and there might also be thickening of the small vessels embedded in the surrounding fibrous tissue.

In one case the presence of inflammatory cells in the node and the mild inflammation in the adherent pericardium indicated the possibility that an extrinsic inflammatory lesion was responsible for the localized reactive lymphadenitis. However, whether the lesion takes origin in a non-specific mediastinitis, in some form of chronic vascular obstruction, or from some other local reaction cannot be determined until a large series is studied as a separate entity.

COMPARISON OF HYPERPLASTIC MEDIASTINAL LYMPH NODES AND THYMOMAS

Except for the instances in which thymomas may be associated with myasthenic symptoms, there is little difference in the early clinical symptoms caused by the two lesions. Thymomas not associated with myasthenia gravis and localized enlargement of mediastinal lymph nodes are both characterized clinically by the appearance of a mass, usually detected by routine roentgenologic examination, occurring in a young person without symptoms. Signs of local extension may occur in

patients with thymomas, but since localized lymph node hyperplasia of the mediastinum is a non-neoplastic lesion, it remains encapsulated and restricted in its growth potential. More difficulty in gross differentiation may occur in those instances in which mediastinitis is associated with the lymphoid hyperplasia causing adhesions of the capsule.

The essential differences between the two lesions are histologic. The confusion with thymomas results from the hyaline change in the center of the lymphoid follicles superficially resembling Hassall's corpuscles. However, reticulum stains and numerous sections show the fundamental anatomical pattern of the mass to be that of a hyperplastic lymph node undergoing certain sclerosing alterations. Further, since the reaction is non-specific, it may be expected that the lesion, unlike thymomas, may be noted in lymph nodes in other regions. For example, nodes removed from a patient with bronchogenic carcinoma showed a similar reaction (Fig. 17). This may explain the puzzling reports of tumors believed to be thymomas occurring in unusual locations within the chest.

Discussion

The significance of these observations lies chiefly in their usefulness as a means of diagnosis and as a tool for further analysis of the tumors discussed.

There are several interesting problems introduced by this study.

(a) The Endocrine Nature of the Epithelial Cells Noted in Thymomas Removed from Patients with Myasthenia Gravis

As noted in this series, the regularity of organization of pale epithelial cells around vessels in thymomas from myasthenic individuals seems almost prophetic of the symptoms to be expected. A series much larger than 27 cases, however, must be studied in order to ascertain how strictly these observations may be applied. Castleman[6] has now verified the fact that these criteria are applicable in at least 75 per cent of the cases at the Mayo Clinic and in his own series. It would seem, therefore, that they can be offered safely, not as an absolute sign for histologic diagnosis of tumors in myasthenic individuals but, as in many other examples of endocrine tumors, as an indication of the nature and functioning state of the cells which are concerned.

It would be helpful to correlate the histologic changes in the tumor over a period of time with remissions of the patient, both those remissions which occur spontaneously and those which are produced artificially by prostigmine therapy. In this series, there is a difference in the average age between myasthenic and non-myasthenic patients with thymomas; the patients without symptoms comprised an older

age group. A very careful exploration of the past history of this group may reveal some symptoms heretofore believed unrelated to the tumor and a clue as to whether the pale spindle cells noted in the tumors removed from them are not, in reality, a primitive or resting stage of the functioning cells.

Some relationship between duration and histologic appearance is indicated in this series by the one case in which an interval of 4 years between biopsies showed a change from a predominantly lymphoid appearance to a tumor mostly composed of spindle cells. Hence, further study may profitably be directed, not toward cytologic classification of the tumors, but toward understanding the histologic alterations occurring in them over periods of time.

(b) The Term Thymic Carcinoma

The term thymic carcinoma has often been applied to ordinary thymomas in patients with myasthenia gravis or to tumors resembling seminomas. There may be tumors which properly can be designated thymic carcinoma; but eliminating those which are clearly thymomas of the classical type, it is probable that most thymic carcinomas, as in this study, are more closely related to seminomas than to thymomas. Of course, it is not unreasonable to suppose that epithelial cells of the thymus may become malignant under certain circumstances, and that, when they do, they need not necessarily engage in functional activity. Nevertheless, it is significant that there are no reported cases of distantly metastasizing thymomas in patients who have had myasthenia gravis.

(c) Origin of Mediastinal Seminomas

As already discussed, the clinical similarity and the histologic evidence of teratomatous transitions or inclusions in those cases designated thymic carcinoma relate them to teratomas rather than to thymomas. Because of this interrelationship, the problem of their origin is linked with the histogenesis of teratomas.

It has been observed that some seminomas arise in the thymus gland, particularly in the cystic thymus; but, as with parathyroid adenomas under similar circumstances, this is not adequate basis for enlisting the thymus itself as the histogenetic factor. Non-specificity is further indicated by the fact that seminomas and teratomas arise elsewhere while thymomas do not.

It is quite possible that the site of origin may be both thymus and mediastinum, but that the tissue of origin in either case is the same. This requires some speculation concerning the possibility of inclusions within the thymus. Using the work of Norris[13] as supportive evidence,

Schlumberger[14] postulated that the ectodermal inclusions together with degeneration of Hassall's corpuscles may initiate production of tridermal tumors. However, in reviewing the embryos on which Norris based his interpretation concerning the ectodermal inclusions within the descending thymus, I have noted considerable latitude for other concepts of the histogenesis of Hassall's corpuscles. In fact, confirmation of all possible theories requires more supportive observation. One may reason equally that germ cells wandering off from their usual path in the embryonic era may be included in an anomalous thymus and later give rise to these tumors, or that certain primitive cells under proper environmental conditions may show unusual potential toward germinal tumors.

(d) Use of the Term Seminoma

If the words seminoma or dysgerminoma are applied to analogous tumors arising in the mediastinum, it indicates the identity of one with the other. Friedman's[11] very pertinent term germinoma commits one to the wandering germ cell theory concerning their origin. Therefore, to avoid coinage of a new and imperfect designation, the term seminomatous tumor is tentatively suggested in order to call forth a prompt mental image of the general class of tumors while allowing for the probability that the location of the particular example, whether pineal gland, mediastinum, testis, or ovary, may have some differentiating influence, both histologically and clinically.

(e) Pathogenesis of Localized Hyperplasia of Mediastinal Lymph Nodes

The pathogenesis of localized hyperplasia of the mediastinal lymph nodes can be studied only after more examples have been properly separated from thymomas and analyzed in respect to associated clinical and histologic findings. As with non-specific lymph node reactions elsewhere, this localized mediastinal enlargement is probably a reaction to a variety of stimuli. In one case the cause of the enlargement was associated pleuritis and pericarditis. The prominent vascular sclerosis suggests also a congestive process, possibly due to obstruction or increased venous tension.

Conclusions

No metastasis occurred in a group of 27 thymomas. Thymomas, therefore, are considered to be generally non-metastasizing but locally extending tumors.

In this series, true thymomas are classified simply into two histologic subgroups related to the presence or absence of symptoms of myasthenia gravis in the patient. Each example is generally a composite of various elements, typical of the group, in varying proportions:

(a) Thymomas in patients without myasthenic symptoms are characterized by stages of lymphoid proliferation, spindle cell proliferation, or stromal proliferation.

(b) Thymomas in patients with myasthenia gravis are characterized by the appearance of large pale epithelial cells, loosely mixed with lymphocytes, and often arranged in cords or clusters around vessels.

The close correlation of the symptoms of myasthenia gravis with the appearance in the thymoma of epithelial cells around vessels suggests an endocrine function for the type-cell.

In this series, most of the cases previously diagnosed thymic carcinoma proved to be seminomatous tumors of the mediastinum. Eleven examples are discussed. They may be differentiated from thymomas by the histologic characteristics of the type cell, by the frequently accompanying granulomatous reaction, by their radiosensitivity, and by their evident malignancy.

Five cases of localized hyperplasia of mediastinal lymph nodes are reported. The lesion is a non-specific reaction, histologically benign, and characterized by alterations of the germinal centers simulating Hassall's corpuscles. It is frequently misdiagnosed as thymoma, both by surgeons and by pathologists.

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LEGENDS FOR FIGURES

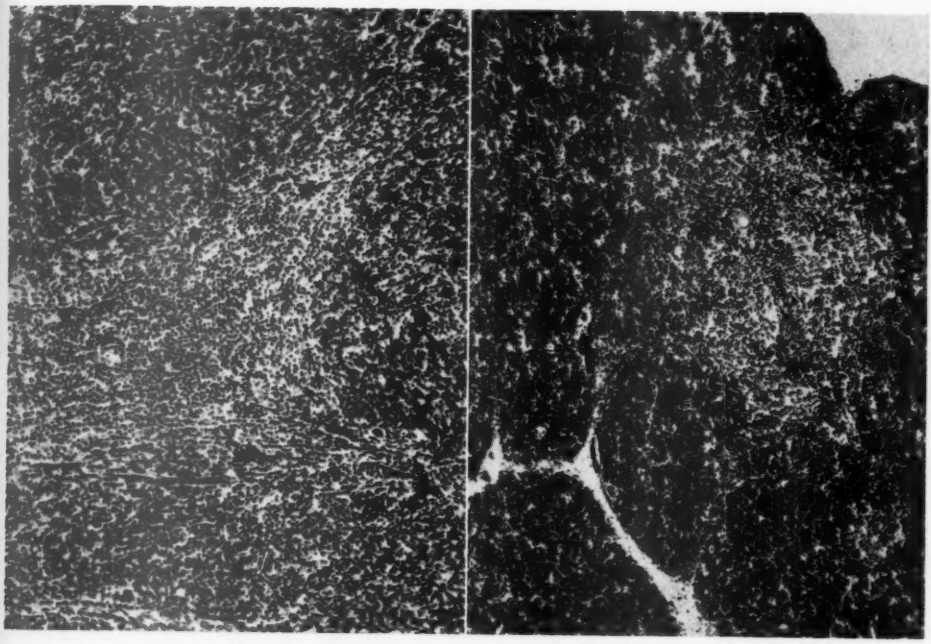
All sections were stained with hematoxylin and eosin unless otherwise stated.

FIG. 1. Armed Forces Institute of Pathology Accession No. 176626. Thymoma not associated with myasthenia gravis, showing lymphoid proliferation. The lobular arrangement and absence of Hassall's corpuscles may be noted. × 125.

FIG. 2. A.F.I.P. Acc. 186567. Thymus gland in an anencephalic infant showing predominance of lymphoid elements and paucity of Hassall's corpuscles. × 75.

FIG. 3. A.F.I.P. Acc. 298252. Thymoma not associated with myasthenia gravis, showing proliferation of spindle cells. Of note are the diminution in lymphoid lobules, absence of Hassall's corpuscles, and microcystic spaces within septa. × 75.

FIG. 4. A.F.I.P. Acc. 178650. Thymus gland from an infant with scurvy showing atrophy, replacement by spindle cells, vascularity of medulla, and atypical, scarce Hassall's corpuscles. × 125.



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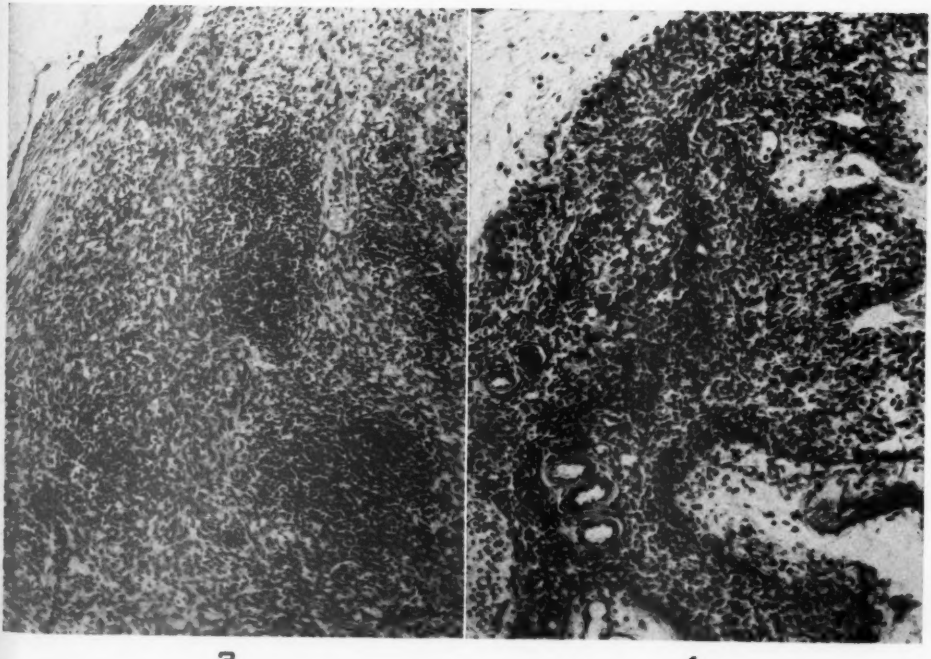

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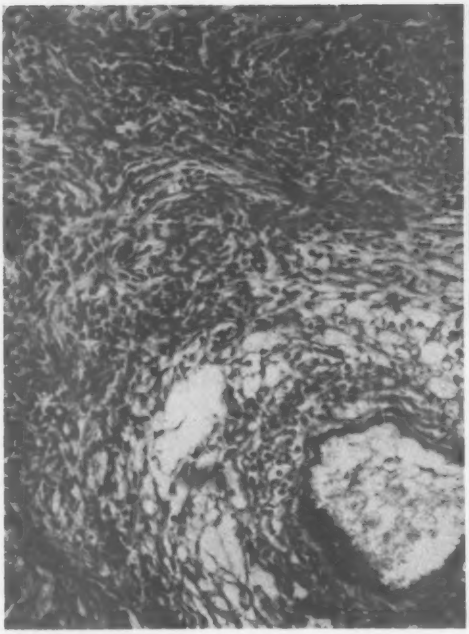

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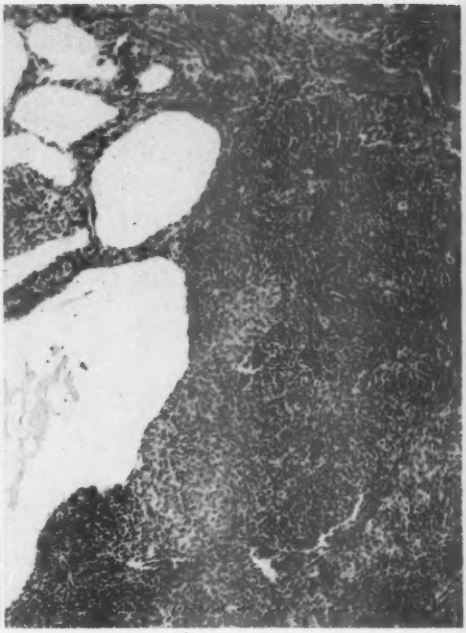

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FIG. 5. A.F.I.P. Acc. 338795. Thymoma not associated with myasthenia gravis. Interlobular septum showing proliferation of spindle cells and formation of cystic spaces. There is an intermixture of lymphocytes and spindle cells in the remaining lobule. × 178.

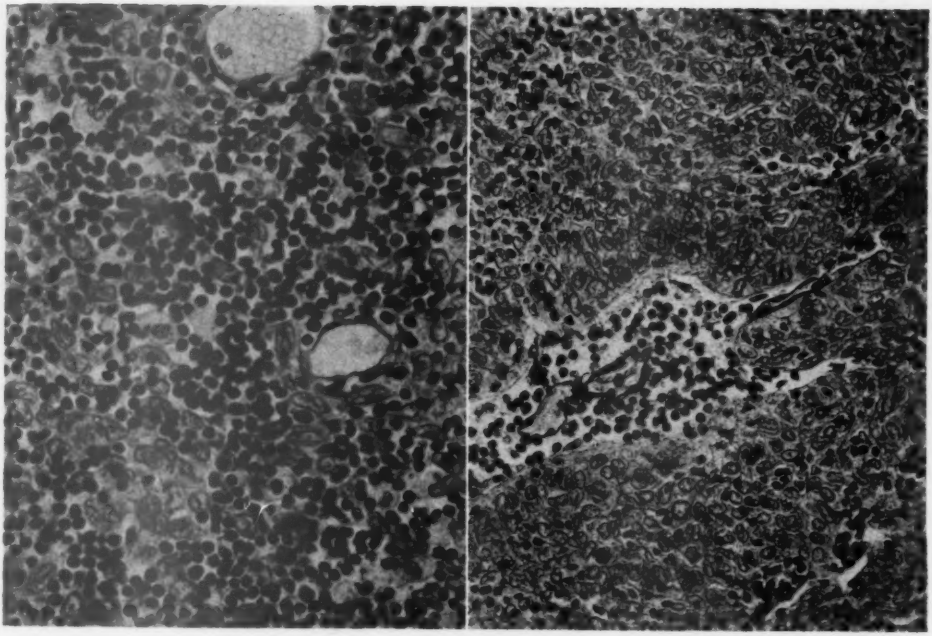
FIG. 6. A.F.I.P. Acc. 520728. Thymoma not associated with myasthenia gravis. Pale spindle cells dominate the lobule. Cystic spaces are associated with the pale spindle cells. Cellularity and vascularity of the tumor and increased fibrosis of the septum may be noted. × 100.

FIG. 7. A.F.I.P. Acc. 520726. Thymoma associated with myasthenia gravis. A characteristic field shows intermixture of large epithelium-like cells with lymphocytes, succulent pulp, and thin-walled vessels encircled by large pale cells. × 453.

FIG. 8. A.F.I.P. Acc. 541010. Thymoma associated with myasthenia gravis. The epithelial cells dominate this field and lymphocytes are limited to sinusoid-like channels. The epithelial cells form solid sheets around the vessels. A central capillary may be noted with a surrounding lymphatic. Periodic acid-Schiff's (PAS) stain. × 290.

FIG. 9. A.F.I.P. Acc. 541010. Thymoma associated with myasthenia gravis. A central capillary and lymphatic are surrounded by cords of epithelial cells in an endocrine-like pattern. PAS stain. × 405.

FIG. 10. A.F.I.P. Acc. 541010. Thymoma associated with myasthenia gravis. The epithelial cells exhibit a tendency to whorl formation and transitions to Hassall's corpuscles. At the periphery some are intermixed with lymphocytes in the more typical manner. × 450.



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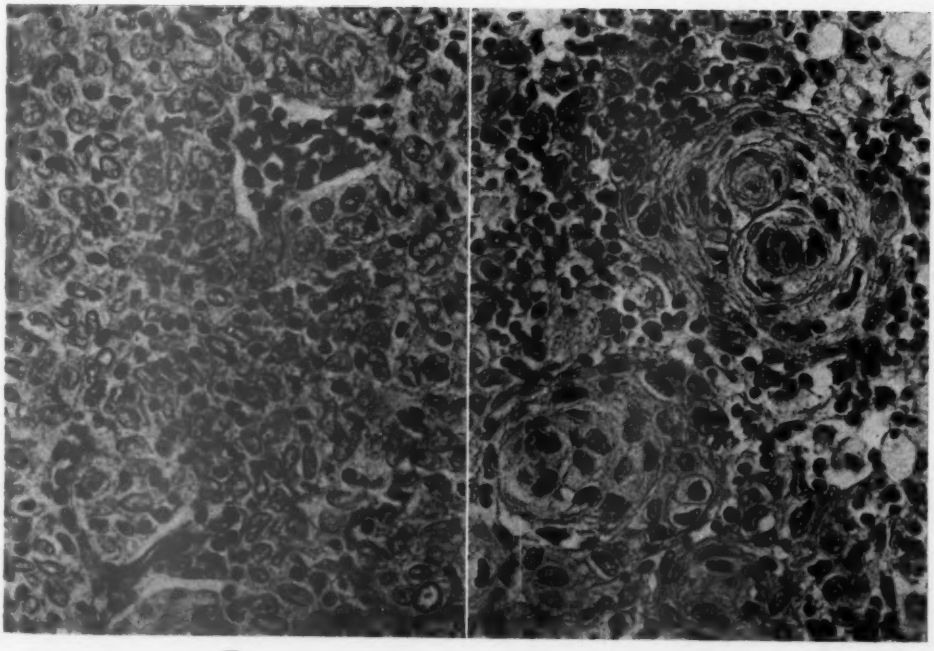

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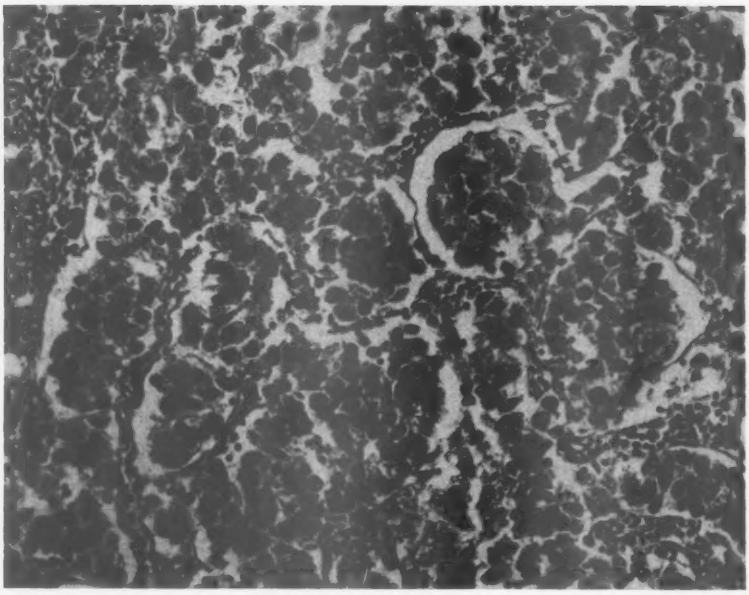

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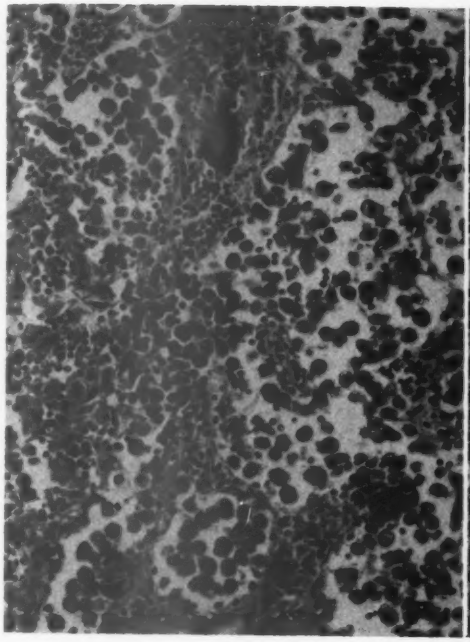
FIG. 11. A.F.I.P. Acc. 528347. Seminomatous tumor of mediastinum. Clusters of dark round cells within acini. Lymphocytes outline the fine connective tissue strands of the stroma. × 220.

FIG. 12. A.F.I.P. Acc. 320877. Seminomatous tumor of mediastinum. The neoplastic cells are loosely clumped within acini. The stroma is accentuated by reticuloendothelial reaction, giant cells, and increase in connective tissue. × 185.

FIG. 13. A.F.I.P. Acc. 167385. Seminomatous tumor of mediastinum. The granulomatous reaction may sometimes dominate the field and obscure the presence of the tumor. There are small cords of tumor cells within the vascular space. × 275.

FIG. 14. A.F.I.P. Acc. 528347. Seminomatous tumor of mediastinum. A teratoid inclusion, showing glands and intervening cells resembling poorly differentiated nervous tissue. This was adjacent to the main tumor which resembled that shown in Figure 11. × 105.



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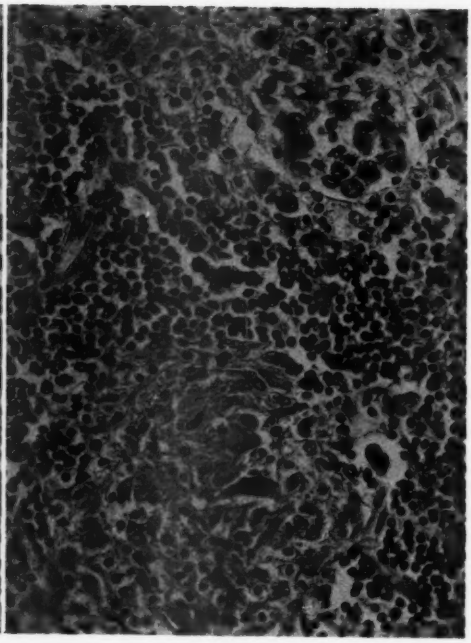

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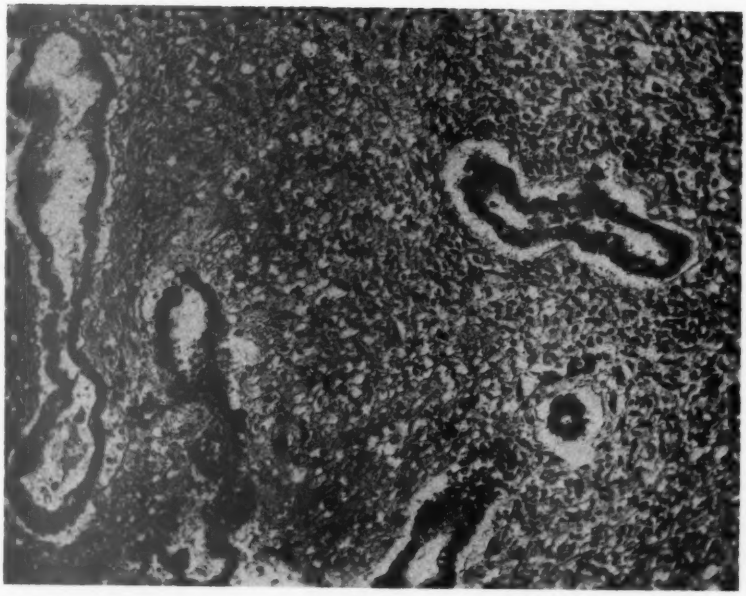

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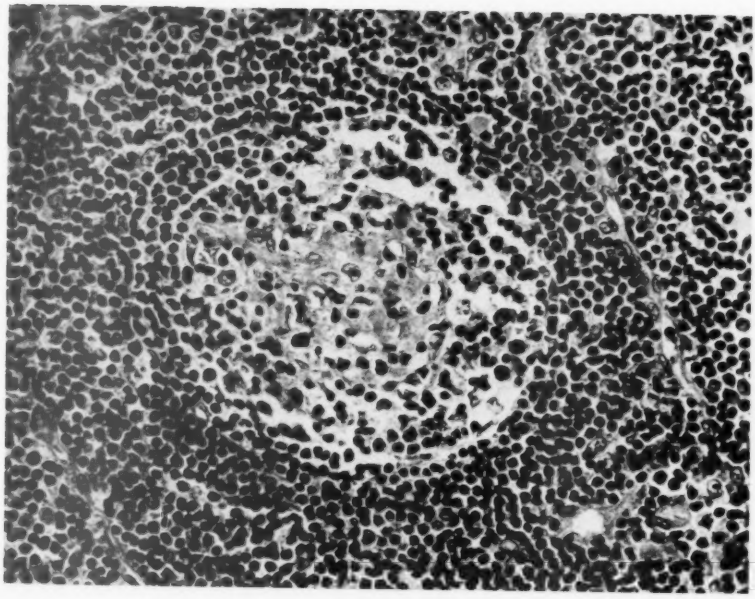
FIG. 15. A.F.I.P. Acc. 523088. Localized hyperplasia of a mediastinal lymph node, showing hyaline alteration of the germinal centers of the follicles. Inflammatory cells may sometimes be noted in the pulp. × 330.

FIG. 16. A.F.I.P. Acc. 484088. Localized hyperplasia of a mediastinal lymph node. Follicles are converted to hyaline masses superficially resembling Hassall's corpuscles. Sinusoids show similar alteration. Septa and lobular pattern of the thymoma are not seen. × 112.

FIG. 17. Reactive hyperplasia of tracheobronchial nodes in a patient with bronchogenic carcinoma. This non-specific reaction resembles that shown in Figure 16 in mediastinal nodes misdiagnosed as thymoma. × 112.



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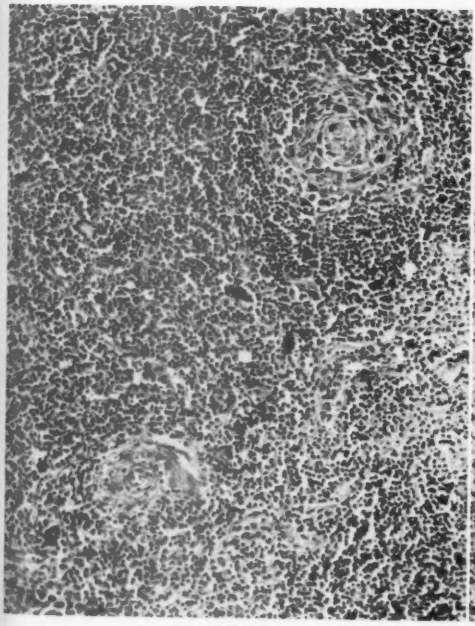

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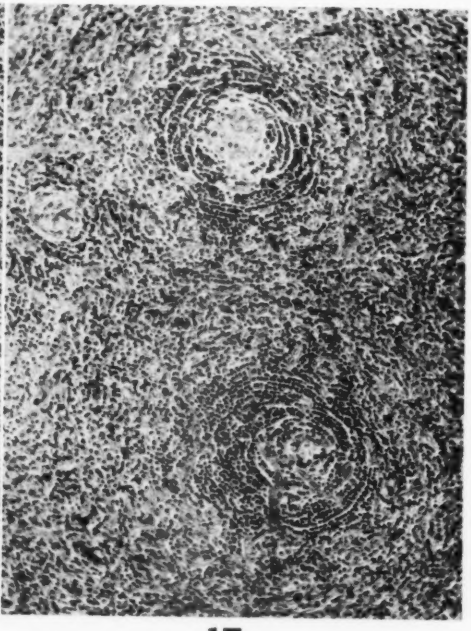

17

HISTOCHEMICAL OBSERVATIONS ON AN ALVEOLAR SOFT-PART SARCOMA WITH REFERENCE TO HISTOGENESIS *

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In 1952, Christopherson, Foote, and Stewart[1] reported a group of 12 soft tissue neoplasms which they designated as alveolar soft-part sarcomas. The actual histogenesis of these lesions could not be determined, although their association with skeletal muscle was a constant finding. Morphologically, they were characterized by large, round, oval, and polyhedral cells with rather abundant cytoplasm, which were acidophilic and occasionally contained fine granules and vacuoles. Eccentric nuclei were variable. Binucleate, hyperchromatic, and anaplastic forms and occasional mitotic figures were evident. The tumor cells were arranged in an alveolated or organoid pattern with approximation to delicate endothelium-lined spaces. That the former configuration might represent artifact produced by the loss of central collections of tumor cells was readily admitted by these authors. Yet, because of this constant structure and lack of evidence of histogenesis, they believed that the descriptive, non-commital term alveolar soft-part sarcoma was warranted. Metastases were present in approximately one half of the cases, although there were no histologic features which enabled one to predict such an event.

Lesions of identical appearance have been considered by some as malignant variants of the more common granular cell myoblastoma.[2-5] The close similarity of individual cells of the alveolar soft-part sarcoma to those of the latter was noted by Christopherson and associates.[1] However, Ross, Miller, and Foote[6] have stated that those neoplasms designated as malignant granular cell myoblastoma could not be irrefutably considered as such since their descriptions failed to indicate a transformation from benign to malignant variants. They found only 4 cases which they accepted as malignant granular cell myoblastomas, to which they added 3 examples. The rejected cases were considered as alveolar soft-part sarcomas.

Another group of neoplasms bearing a marked morphologic and biologic similarity to the alveolar soft-part sarcomas was described previously by Smetana and Scott[7] and designated as malignant nonchromaffin paragangliomas. Although one half of these lesions were observed in the thigh, a site not usually associated with paraganglionic

* Received for publication, September 17, 1955.

tissue, they cited a communication from Johnson[8] describing paraganglion-like glomera about the femoral vessels. Similar tumors were found in the neck and retroperitoneal tissues.

There are, therefore, several groups of soft tissue tumors which from their nomenclature appear unrelated, but because of morphologic and biologic similarities merit further investigation to establish their proper nosologic relationships. Since the opportunity for recognizing the actual derivation of some mesenchymal neoplasms from morphologic study is limited, it was believed that significant information concerning the histogenesis of alveolar soft-part sarcomas might be gained from a thorough investigation of the histochemical reactions of the tumor cells of an example of such a lesion. Further, since similar investigations have been performed previously on the benign granular cell myoblastoma,[9,10] a comparison between the results might offer specific information concerning the relationship between the classical granular cell myoblastoma and its so-called malignant variant.

Material and Methods

The alveolar soft-part sarcoma utilized in this study was obtained at necropsy from a white man, 26 years old, who first noticed painful swelling in the lateral aspect of the right thigh 7 years before death. Local excision had been performed on several occasions but recurrence followed, and at the time of death the tumor measured 15 by 9 by 8 cm. Its outer surface was smooth and covered, in part, by a moderately thin, white capsule which, in several areas, was attached to but not infiltrating the lateral muscles of the thigh. The cut surface was yellow-tan, moderately convex and firm, with irregular areas of hemorrhage in the periphery. Metastases of similar appearance were found in the dorsal vertebrae, mediastinal lymph nodes, liver, and both lungs. The microscopic appearances of the primary and secondary growths were similar, presenting the morphologic features of the alveolar soft-part sarcomas described previously (Figs. 1 and 2).

Blocks of tumor from the primary and secondary sites were fixed in 10 per cent neutral formalin, dehydrated, and infiltrated with paraffin in the usual manner. Some were placed in Bouin's fluid after fixation in formalin, followed by immersion in pyridine for a similar period at 60° C., and then treated with 2.5 per cent potassium dichromate for 48 hours, as in the Baker pyridine extraction procedure, before dehydration and paraffin infiltration. Other blocks of formalin-fixed tissue were placed directly in 2.5 per cent potassium dichromate for 24 hours before dehydration and infiltration.

Blocks of spinal cord containing posterior nerve roots from this case and a classical benign granular cell myoblastoma of the tongue were similarly treated. Sections of these were stained simultaneously with those prepared from the alveolar soft-part sarcoma. In addition, 2 examples of benign non-chromaffin paraganglioma—one from the mediastinum and the other from the retroperitoneal region—which had been previously fixed in formalin and infiltrated with paraffin also were studied by techniques applicable to tissue processed in this manner.

All paraffin sections were taken to water in the usual manner except when otherwise indicated by a specific technique. Frozen sections were cut at 10 μ from wet formalin-fixed tissue with and without post chromation or pyridine extraction.

The following procedures were performed according to the methods described by Lillie[11]:

A. Lipid Methods

1. Oil red O and Sudan black B, the supersaturated isopropanol method. Paraffin sections were stained for 3 hours and frozen sections for 15 minutes at room temperature.
   (a) After immersion of sections in pyridine for 24 hours at 60° C.
   (b) After immersion of sections in chloroform-methanol (equal parts) for 1, 2, and 4 days at 60° C.
   (c) Directly on sections prepared by en bloc pyridine extraction.
   (d) Directly on sections followed by immersion in acetone for 2 minutes and then restained.
   (e) Following the periodic acid-Schiff (PAS) procedure.
2. The peracetic acid-Schiff reaction for the demonstration of ethylenic linkages. Control sections of skin containing hair.
3. The direct Schiff reaction. Sections treated with the Schiff reagent for 72 hours and then taken through reducing rinses as in the PAS procedure (*infra vide*).
4. Plasmal reaction of Feulgen-Voigt, Hayes modification.
5. Carbonyl reaction of Ashbel-Seligman.
6. Lillie's variant of the Weil-Weigert myelin method. Sections were decolorized until red blood cells were gray but myelin sheaths black.
7. Schultz's method for cholesterol and cholesterol esters.
8. Baker's acid hematin modification of the Smith-Dietrich procedure.
9. Examination by polarized light.

B. The Periodic acid-Schiff (PAS) Methods

1. PAS method with alum hematoxylin counterstain performed under the following conditions:
   (a) After digestion of sections with barley malt diastase (Fisher Lot no. 542566), 0.1% in saline-phosphate buffer, pH 6, for 1 hour at 37° C. Glycogen-laden liver sections utilized as enzyme control.
   (b) After digestion with trypsin (Difco, Lot no. 426009), 0.1% in M/100 phosphate buffer at pH 7.6 at 37° C. for 2, 4, and 18 hours. Fibrin thrombus utilized as enzyme control.

(c) After digestion with pepsin (Difco, Lot no. 419687), 0.1% in N/10 HCl for 2, 4, and 18 hours at 37° C. Fibrin thrombus utilized as control.

(d) After acetylation with acetic anhydride: pyridine (16:24) for 4 hours at room temperature.

(e) After digestion with testicular hyaluronidase (Nutritional Biochemicals Corp. Lot no. 9544, 150 TRU/mg.) 150 units per cc. in sodium acetate-acetic acid buffer, pH 5.5, at 37°C. for 1 hour. Sections of cockscomb, stained by the Rinehart-Abul-Haj method and similarly treated, utilized as enzyme control.

(f) After extraction procedures as described in A-1 a, b, and c.

2. Allochrome method.

C. Protein Methods

1. Millon's reaction.
2. Ninhydrin-Schiff's procedure for demonstration of amino groups.
3. Diazo-safranin coupling procedure.
4. Feulgen nucleal method.

D. Basic Dyes and Metachromasia Methods

1. Thionine, 0.05% in M/100 acetate buffer at pH 4 for 30 minutes. Sections differentiated in alcohol as well as mounted directly from water.
2. Crystal violet amyloid method.

E. Other Procedures

1. Hematoxylin and eosin.
2. Ferric ferricyanide method.
3. Wilder's reticulum method.
4. Phosphotungstic acid hematoxylin after mordanting sections in saturated sublimate for 3 hours at 72° C.
5. Masson's trichrome.

Discussion

Some of the tinctorial features of the alveolar soft-part sarcomas, malignant non-chromaffin paragangliomas, and malignant granular cell myoblastomas have been noted previously, although thorough investigation of these properties has not been performed. Christopherson and associates[1] observed sudanophilic droplets within the cytoplasm of tumor cells of the alveolar soft-part sarcomas in apparently non-viable areas. Vacuoles in other portions were not sudanophilic and did not stain with osmic acid or carmine. Glycogen was found in the one example in which glycogen stains were performed, although the technique utilized was not indicated. The tumor cells of the malignant non-chromaffin paragangliomas reported by Smetana and Scott[7] possessed a faint reddish tint when stained with oil red O, as well as occasional larger sudanophilic droplets. A reddish hue to the cytoplasm was observed when sections were stained by the PAS method. The relationship between the sudanophilic and Schiff-positive material was not mentioned and further exploration into the nature

of these reactions was not undertaken. Sudanophilic and carminophilic droplets were noted in areas of degeneration in a malignant granular cell myoblastoma by Ross and associates.[6] Another example revealed carminophilic droplets to be present also in viable areas. Therefore, one may conclude from the literature that the cells of these soft tissue tumors contain sudanophilic material, particularly in degenerating areas. A positive PAS reaction has been noted in those lesions designated as malignant non-chromaffin paraganglioma and glycogen has been said to be present in one example of an alveolar soft-part sarcoma in which this material was investigated.

As seen in Table I, the granules found in many of the tumor cells of the alveolar soft-part sarcoma studied are sudanophilic (Figs. 3 and 4). This property is present in the cells in viable as well as degenerating areas of the primary tumor and its metastases. That the sudanophilia observed is due to lipid is confirmed by the ability to restain the granules with oil red O after decolorization with acetone. This procedure indicates the physical character of the staining and is characteristic of lipids stained with such dyes as oil red O. On the other hand, the sudanophilia is resistant to the usual solvents employed in the preparation of paraffin sections, for the reaction was as intense in sections prepared in this manner as in those cut from frozen tissue. Such sudanophilia has been noted with a number of lipid substances, including ceroid, lipofuscin, lutein, unsaturated fats, myelin, and cerebrosides.[11] However, their lack of color in unstained sections and, particularly, absent cyanophilia with basic dyes and negative direct Schiff's and peracetic acid-Schiff's reactions tend to eliminate the first four mentioned as being responsible for the sudanophilia observed. The granules appear not to be comprised of phospholipids, since reactions to detect such material have been negative. The reactions in regard to the type of lipid encountered within the granules appear to be more consistent with those of cerebrosides. The latter have been observed previously not to retain the iron hematoxylin of myelin methods,[11] and their reaction with acid hematin might be considered equivocal. The cerebroside kerasin contains lignoceric acid[12] which is saturated and would account for the negative peracetic acid-Schiff and direct Schiff reactions. Uzman[13] has noted that kerasin in Gaucher's disease is resistant to extraction with warm chloroform and methanol. A similar situation is obtained with methanol-chloroform extraction of the sudanophilic granules in the cytoplasm of tumor cells of the alveolar soft-part sarcoma after chromation of tissue, although extraction was accomplished in tissue not

TABLE I

Results of Reactions on Granules of Alveolar Soft-Part Sarcoma, Granular Cell Myoblastoma, Peripheral and Cord Myelin

Procedure	Alveolar soft-part sarcoma				Granular cell myoblastoma				Peripheral myelin				Cord myelin			
	Pc	Pnc	Fc	Fnc	Pc	Pnc	Fc	Fnc	Pc	Pnc	Fc	Fnc	Pc	Pnc	Fc	Fnc
Oil red O	+	+	+	+	+	+	+	+	+	±	+	+	+	±	+	+
Pyridine extractions	−	−	−	−	±	±	±	±	−	−	−	−	−	−	−	−
Chloroform-methanol	+	?	+	+	+	+	+	+	+	−	+	+	−	−	−	−
Myelin method (iron hematoxylin)	−	−	−	−	−	−	−	−	+	±	+	+	±	+	+	+
Pyridine extractions									−	−	−	−	−	−	−	−
Acid hematin	−	−	−	−	−	−	−	−	+	±	+	+	+	±	+	+
Pyridine extractions									−	−	−	−	−	−	−	−
Plasmal			−	−			−	−			+	+			+	+
Carbonyl			−	−			−	−			+	+			+	+
Peracetic acid-Schiff	−	−	−	−	−	−	−	−	+	±	+	+	+	±	+	+
Direct Schiff	−	−	−	−	−	−	−	−	±	±	±	±	±	±	±	±
Birefringence	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+
Schultz	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Periodic acid-Schiff	+	+	+	+	+	+	+	+	+	±	+	+	+	−	+	+
Pyridine extraction	±	±	±	±	+	+	+	+	±	±	±	±	−	−	−	−
Chloroform-methanol	+	+	+	+	+	+	+	+	+	±	+	+	−	−	−	−
Diastase	+	+	+	+	+	+	+	+	+	±	+	+	+	−	+	+
Acetylation	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−
Oil red O	Orange-red and purple				Orange-red				Orange-red				Orange-red			
Hyaluronidase	+	+	+	+	+	+	+	+	+	±	+	+	+		+	+
Pepsin	+	+	+	+	+	+	+	+	+	±	+	+	+		+	+
Trypsin	+	+	+	+	+	+	+	+	+	±	+	+	+		+	+
Rinehart-Abul-Haj	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Millon	?	?	?	?	?	?	?	?	−	−	−	−	−	−	−	−
Diazo-safranin	?	?	?	?	?	?	?	?	−	−	−	−	−	−	−	−

Diazo-safranin	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Ninhydrin-Schiff	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feulgen nucleal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ferric ferricyanide	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thionine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	M
Allochrome	Red-purple and pink-tan			Red-purple and pink-tan						Red-purple (neg. Pnc)						Red-purple (neg. Pnc)						Red-purple (neg. Pnc)	
Phosphotungstic acid hematoxylin	No striations			No striations																				
Masson	Brown			Brown																				
Hematoxylin and eosin	Eosinophilic			Eosinophilic																				
Argyrophilia	—	—	—	—	—	—	—			—	—	—	—			—	—	—	—			—	—	—

Pc = Paraffin, chromed. Fc = Frozen section, chromed. M = Metachromatic.
Pnc = Paraffin, not chromed. Fnc = Frozen section, not chromed. ? = Doubtful or very faint.

subjected to chromation. This is similar to the observations of Morrison and Hack[12] on pure kerasin. Such findings in the light of doubtful or faintly positive protein tests might well be accounted for by the fact that the lipid in Gaucher's disease is bound to protein as Uzman has indicated, whereas that within the granules of the alveolar soft-part sarcoma does not possess such linkage; or, if protein is present, it is scant in amount or this glycolipid is adsorbed to the protein. The latter arrangement has been considered characteristic of cerebrosides in the natural state.[14]

The positive PAS reaction of the cytoplasmic granules in the alveolar soft-part sarcoma (Figs. 1 and 5) might be explained as the result of the carbohydrate moiety of the cerebroside. Indirect evidence to support this contention is offered by the following observations. That the PAS reaction is not due to glycogen is indicated by the resistance of this reaction to diastase digestion. Metachromasia was not observed and the negative Rinehart-Abul-Haj reaction for acid mucopolysaccharides, as well as the resistance to treatment with hyaluronidase, argue against the possibility of the reaction being due to acid mucopolysaccharide. The prompt blockade offered by acetylation indicates that 1,2 glycols are responsible for the reaction. This observation, in conjunction with the negative peracetic acid and direct Schiff reactions, indicates that the reactive material is not due to epoxide configuration in unsaturated lipid. Although

the PAS reaction parallels the sudanophilia in being resistant to extraction with chloroform and methanol, only moderate reduction in the intensity of this reaction was noted with pyridine extraction. This difference further suggests that some of the cerebrosides or other polysaccharides might be linked to protein and may explain the faint protein reaction occasionally noted. Sequence staining with oil red O and the PAS reaction indicates that for the most part both reactions occur at the same site. However, in some cells the color produced by the PAS reaction appears to be unaltered by that of oil red O. Similarly, the true red color of the oil red O procedure appeared unaffected by the sequence method, particularly in degenerating areas. The loci demonstrating only a positive PAS reaction in the light of the faint protein reactions and decreased resistance to pyridine extraction might be explained as being due to glycoprotein.

One may conclude from the foregoing that the granules observed within the tumor cells of the alveolar soft-part sarcoma studied contain lipid which is most likely in the form of cerebroside, some of which may be adsorbed or linked to protein, and a small amount of polysaccharide, probably bound as glycoprotein.

This study reveals marked tinctorial similarities between the granules of the alveolar soft-part sarcoma and benign granular cell myoblastoma. The results observed with the latter agree with previous investigations reported by Pearse[9] and Bangle.[10] Pearse considered the granules to be comprised of lipoprotein. Evidence for a protein moiety consisted of weakly positive protein reactions and, more significantly, the resistance of the sudanophilia and PAS reaction to extraction of fresh tumor tissue with boiling chloroform-methanol. Although Bangle did not perform protein reactions, he also considered the granules to be lipoprotein or sphingolipid (cerebroside). Both investigators concluded from their histochemical and morphologic studies that the benign granular cell myoblastoma was of neural origin, a concept that has gained wide acceptance since the studies of Fust and Custer[15] in 1949. Although the exact cell of origin of these tumors is not certain, Pearse believed it to be the perineural fibroblast, whereas Fust and Custer considered the Schwann cell to be the responsible cellular element. Recently, Davis and Butt[16] observed identical histochemical features between the granular cell aggregates found in the neurohypophysis and granular cell myoblastomas from other locations. Their studies indicated that these cellular collections within the pituitary body were derived from an autonomous proliferation of oligodendrogliocytes, which are the central nervous system

homologues of Schwann cells. They also considered the cell of origin of the extra-hypophyseal granular cell myoblastomas to be the Schwann cell. Germaine to the chemical similarities between the alveolar soft-part sarcoma and granular cell myoblastoma is the similarity between the granules of the former and myelin, particularly peripheral myelin or that associated with Schwann cells (Fig. 6). The sudanophilia and positive PAS reactions, including the effects of the various extraction procedures employed, are identical. Although myelin, in addition, is birefringent, metachromatic, and colored by the usual myelin methods and the peracetic acid-Schiff reaction, it would not appear unjust to consider that all of the complex components of myelin (proteins, phospholipids, cholesterol, inorganic salts, sulfatide, and cerebrosides) do not have to be represented in tumors of neural derivation. The high cerebroside content of peripheral myelin, which substance was demonstrated within the granules of the alveolar soft-part sarcoma, has been noted. The lack of birefringence might be the result of physical rather than chemical differences between the tumor granules and myelin. These interpretations, based on the chemical nature of the cytoplasmic granules of the alveolar soft-part sarcoma and their similarity to the granules of another not too dissimilar tumor of neural origin, strongly suggest a similar derivation for the alveolar soft-part sarcoma. Although the morphologic features of the alveolar soft-part sarcoma are unlike those of the more common neural tumors, examples of the latter which do not reveal definite atavistic neural features have been noted. The epithelial variant reported by Gore[17] and the case noted by McCormack, Hazard, and Dickson,[18] who were able to follow the development of such a lesion from proliferating Schwann cells, substantiate such a possibility. It is interesting to note that Figure 5 from the latter's case report reveals cytologic and architectural features similar to the alveolar soft-part sarcoma. Neural elements were noted in the alveolar soft-part sarcoma studied, as well as in those reported by Christopherson and associates[1] and the malignant non-chromaffin paragangliomas described by Smetana and Scott,[7] although morphologic evidence for histogenesis from such elements was lacking.

The limited tinctorial studies performed on examples of malignant non-chromaffin paragangliomas[7] appear identical to those observed with the alveolar soft-part sarcoma. Morphologically, there does not appear to be any discernible difference between the two lesions. Investigation of some of the tinctorial properties of two classical "benign" non-chromaffin paragangliomas failed to reveal significant

sudanophilia and PAS-positive granules were sparse. Many of the latter were labile to diastase treatment, indicating their glycogen content. Such information suggests that the alveolar soft-part sarcoma and malignant non-chromaffin paragangliomas are similar, if not identical lesions. If the latter are derived from paraganglionic tissue, the site of origin would, in the light of previous statements, be the neural components of such structures. Smetana and Scott[7] mentioned the possibility that paraganglionic tumors may reveal variations because of their various cellular components.

One hesitates to propose a new name for the alveolar soft-part sarcoma, because that term, in a descriptive sense, possesses merit. The chemical similarities of the lesions investigated to the granular cell myoblastomas are marked. However, it cannot be stated with assuredness that these lesions represent malignant granular cell myoblastomas. Such information may be derived from similar studies performed with benign granular cell myoblastomas revealing malignant transformation as described by Ross and associates.[6] Further, the perpetuation of the term myoblastoma for such lesions appears unwise, since evidence has accumulated indicating that this histogenetic implication is highly tenuous, if not totally incorrect. Therefore, it is considered that the descriptive term alveolar soft-part sarcoma should be retained for these lesions with the full appreciation that they appear to be, from the evidence presented, of neural origin and that their histogenetic differentiation from so-called malignant granular cell myoblastomas and malignant non-chromaffin paragangliomas is extremely difficult and requires further proof.

Summary

The cytoplasmic granules of an alveolar soft-part sarcoma have been characterized histochemically as consisting mainly of cerebroside, some of which may be linked or adsorbed to protein. Lesser quantities of polysaccharide, apparently linked with protein, are evident also. The chemical nature of these granules and the marked tinctorial similarity to the granules of the so-called granular cell myoblastoma and peripheral myelin strongly suggest a neural derivation for such neoplasms.

The descriptive term alveolar soft-part sarcoma is retained, although the results of this study indicate that this lesion is perhaps identical to those described as malignant non-chromaffin paragangliomas and that their relationship to the more common benign granular

cell myoblastoma is closer, at least histogenetically, than their morphologic variations might indicate.

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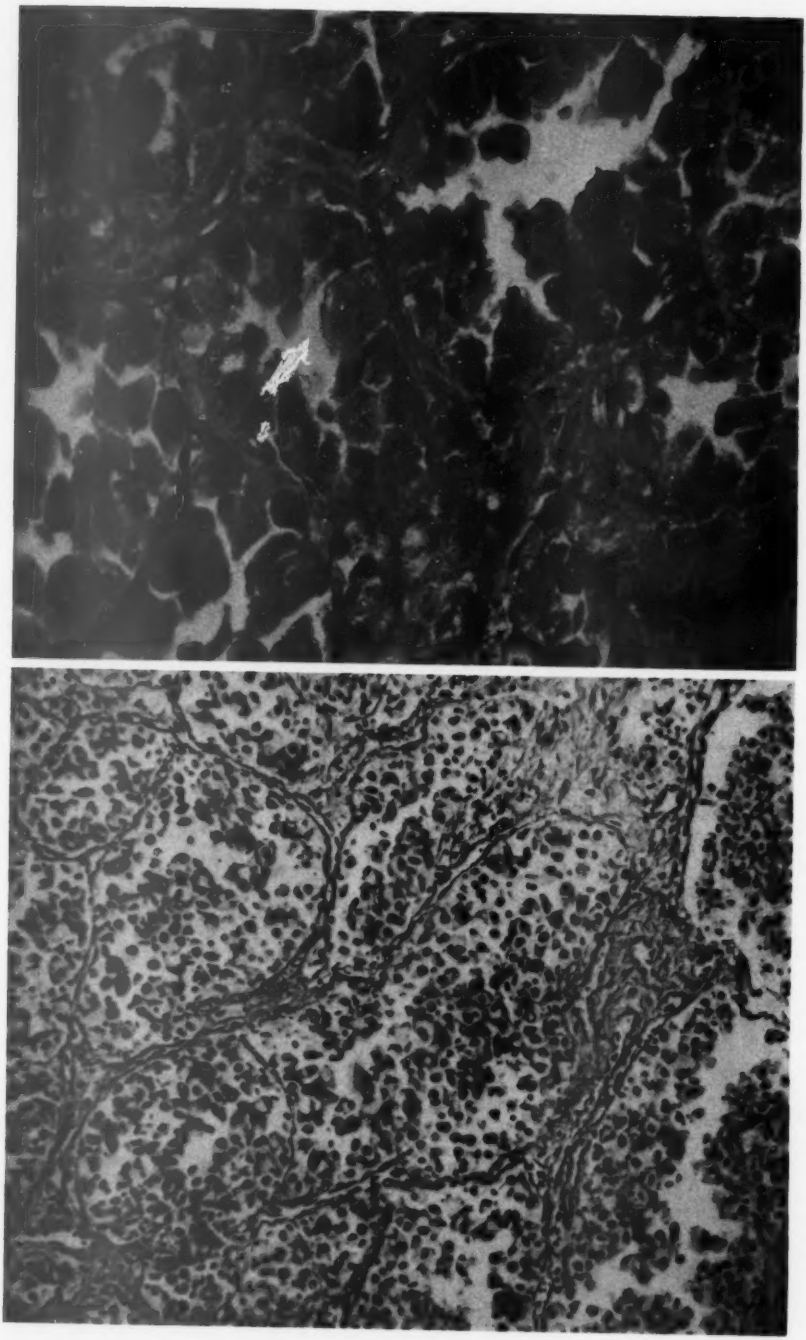
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Section of alveolar soft-part sarcoma stained by the periodic acid-Schiff (PAS) technique demonstrating alveolated masses of tumor cells. Cytoplasm of many appears gray and black due to positive granules. × 200.

FIG. 2. Section stained by Wilder's reticulum method revealing relationship of tumor cells to endothelium-lined spaces. × 125.

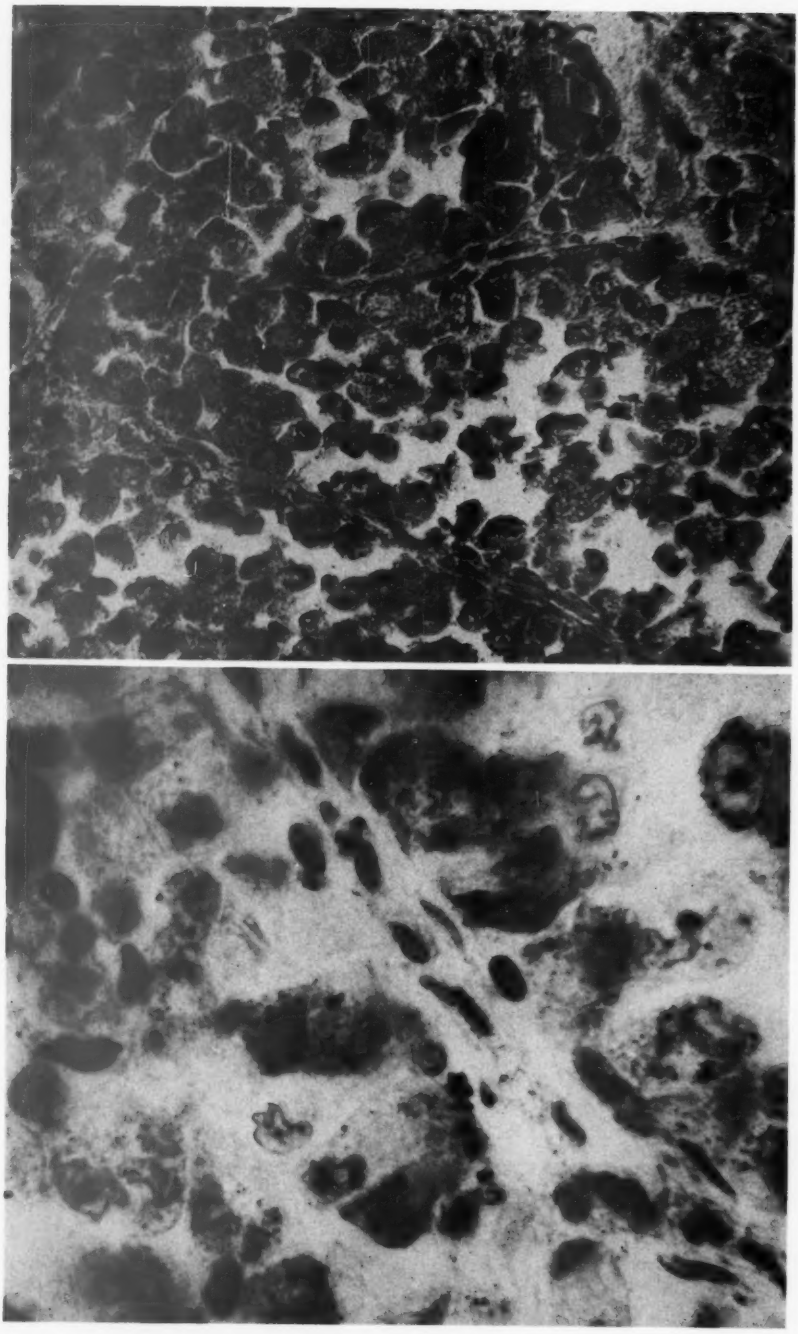


1

2

FIG. 3. Section stained with oil red O. The sudanophilic granules appear black and gray. × 200.

FIG. 4. High-power appearance of sudanophilic intracytoplasmic granules, which appear black and gray. × 900.

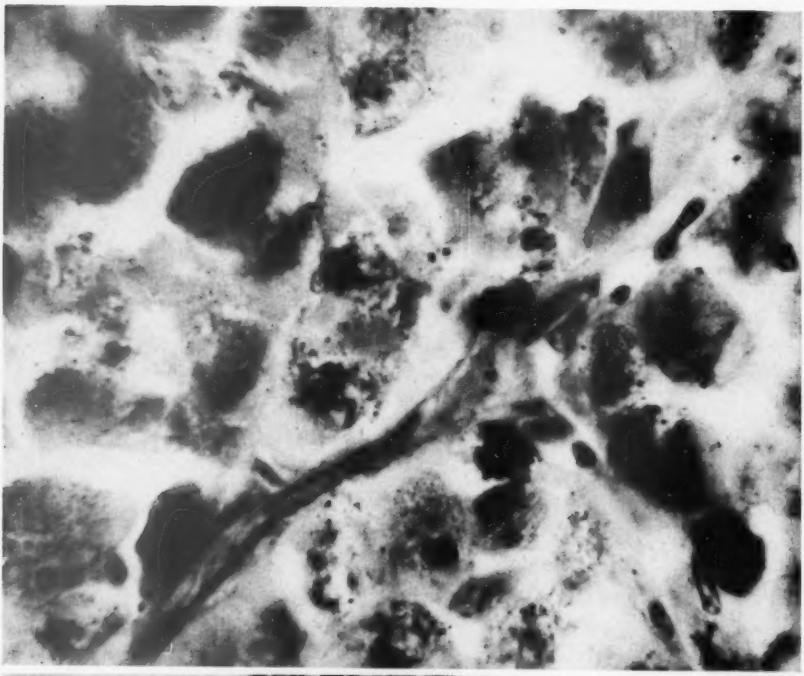


3

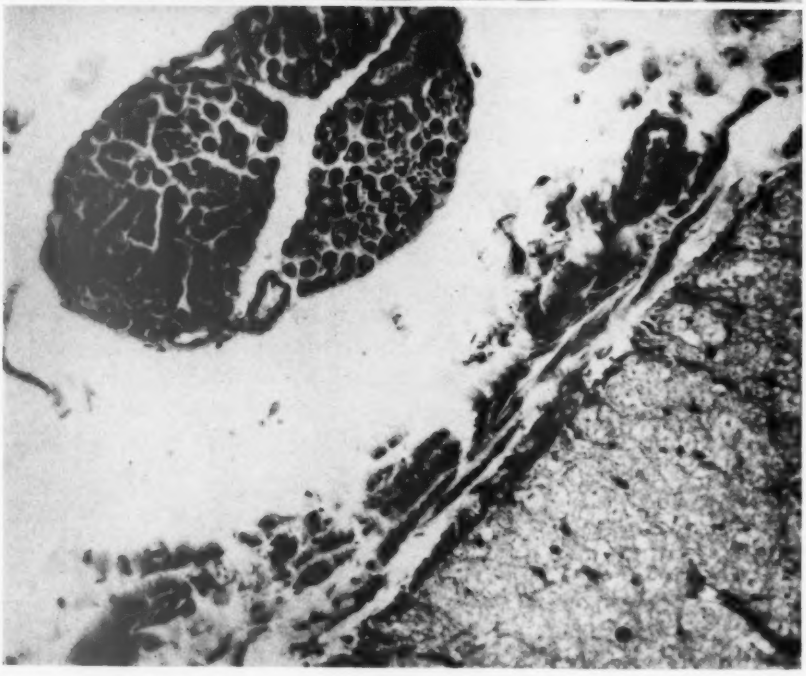
4

FIG. 5. High-power appearance of positive PAS granules within tumor cells of alveolar soft-part sarcoma. × 900.

FIG. 6. Section of cord stained by the PAS method following extraction with chloroform-methanol. The peripheral myelin has resisted extraction and is colored, whereas the PAS-positive material within cord-myelin apparently has been extracted. × 125.



5



6

SILICOSIS: THE TOPOGRAPHIC RELATIONSHIP OF MINERAL DEPOSITS TO HISTOLOGIC STRUCTURES *

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In order to visualize intrapulmonary deposits of silica and silicates, it has been customary to incinerate paraffin sections which are duplicates of the stained sections. The ash remaining following treatment with hydrochloric acid and water is presumed to consist largely, if not entirely, of silica or silicates, or both. Subsequent examination of the incinerated slide under dark-field conditions and comparison with the duplicate stained section give the examiner impressions of the amount of mineral and its approximate disposition in the tissue. However, descriptions of the *exact* position of silica deposits in relation to anatomical structures have not been found in the literature.

The present report concerns a method for demonstrating the exact relationship of intrapulmonary inorganic dust deposits to histologic structures and some of the findings obtained by this method.

METHOD

Silicosis was produced by the intratracheal injection of 40 mg. of silica suspended in 1 ml. of water and also by subjecting rats in inhalation chambers to air containing 25 mg. of silica per cu. m. during 6 hours per day for as long as 15 months. The average particle size of the silica injected intratracheally was 0.8 μ (by optical microscope) while that of the silica in the inhalation chamber measured 0.02 μ (by electron microscope). At various intervals, rats from both groups were sacrificed with ether and the lungs inflated with formalin. The lungs of animals which died of intercurrent infection were similarly prepared.

Carefully selected and marked fields in certain sections of such lungs, stained either with hematoxylin and eosin or with the periodic acid-Schiff technique, were photographed on 2¼" by 2¼" film. Following photography, the coverslips of the slides were removed and the slides placed in an electric furnace for an average period of 3 hours at temperatures between 550° and 600°C. After cooling, the slides were placed in concentrated hydrochloric acid for 30 minutes, washed

* A portion of this investigation was supported by funds from the Johns-Manville Corporation, 22 East 40th Street, New York 16, N.Y.
Received for publication, October 7, 1955.

with water, and allowed to dry. Coverslips were replaced, and the same fields previously photographed were re-photographed at the same magnification but under dark-field conditions. Enlarged prints were made of the negative of the stained section and of the composite negative prepared by carefully superimposing the dark-field negative upon the first negative and then binding these together with transparent adhesive tape. In order to demonstrate the mineral clearly, it was necessary to overexpose the composite print greatly; hence the need of the normal print of the photomicrograph for the finer details.

In addition to the experimental lesions, a small number of fields from sections of the lungs of two asymptomatic human cases of silicosis were studied by this method. One patient was a white man, 77 years old, who died of pulmonary embolism; the other patient, a white man, 72 years of age, died of cor pulmonale with pulmonary emphysema and edema. Pulmonary tuberculosis was present only in the latter patient in whom it was minimal and consisted of a few discrete tubercles.

Altogether, over 300 photomicrographs were studied.

Results

Experimental silicosis: intratracheal injections

The lungs of animals sacrificed 2 months following the intratracheal injection contained numerous small discrete, and larger confluent nodules which were cellular but contained little or no collagen. Some of the discrete nodules were well filled with dense, compact masses of silica (Fig. 3). While a few of these silica masses had smooth contours, many of them had nodular or serrated outlines. The larger nodules frequently exhibited central portions which were devoid of silica and contained only relatively few scattered flocs in the peripheral portions. Air spaces adjacent to some of the silicotic nodules frequently were partially outlined by silica flocs which apparently were in contact with the alveolar walls (Fig. 5). Some of this silica seemed to be extracellular, but in some regions most of the silica was found within lipidic macrophages which often formed solid plugs occluding the alveolar lumen (Fig. 7). In some of the air spaces, punctate extracellular flocs also were present.

In some animals, there was a tendency for silica flocs to aggregate in subpleural regions and within the pleura itself (Fig. 9). In a few regions of confluent nodulation where the adjacent parenchyma showed partial atelectasis and collagenous thickening of the walls, there was diffuse infiltration of interstitial tissues by heavy flocs and

scattered, more finely divided silica. In contrast, the air spaces here contained very little mineral, generally adherent to, or incorporated in, alveolar débris. A somewhat different picture was seen in another subpleural region of diffuse alveolar fibrosis where the air spaces were extensively occupied by cellular débris replete with silica (Fig. 9). Here, also, many smaller discrete flocs were found within the alveolar walls and within the pleura.

In an animal which was sacrificed 1 year following an intratracheal injection of silica, there were several pulmonary collagenous silicotic nodules in which there was little or no mineral (Fig. 11). The extranodular mineral was finely divided and scattered, occupying not only interstitial but also alveolar positions. Some of it tended to outline alveolar walls.

Inhalation Chamber

Exposure to silica dust in an inhalation chamber resulted in pulmonary nodules and other findings very similar to those in the intratracheal group of animals. Some lung sections (rat 1363, exposure 15 months, sacrificed after a holding period of 9 months) contained isolated, discrete, generally rounded nodules in which the pattern due to silica almost completely obscured the histologic pattern (Fig. 13). Here the silica was dense and homogeneous, and the outline of the masses was generally smooth. The parenchyma between the nodules was essentially normal and only very few scattered aggregates of silica were demonstrable external to the nodules. These few aggregates were adherent to alveolar walls.

In contrast, there were sections from another animal (rat 1338, exposure 15 months, sacrificed after a holding period of 7 months) in which the nodules were largely devoid of mineral (Fig. 15). Although these nodules also were rounded, they were not discrete or isolated since adjacent to them there were irregular, smaller nodules connected with the larger by thick, fibrous, alveolar walls. Such secondary nodules were frequently more cellular, less collagenized, and were therefore presumed to be younger. It was not uncommon to find a considerable amount of silica in the younger, secondary nodules although the peripheral portions often were free. Another very striking difference between the patterns of distribution of silica in these two animals was the presence of abundant silica in the parenchyma between the nodules in the second animal, whereas very little was found in this location in the first. Much of the mineral appeared to be extracellular, attached to alveolar walls, but some of it was related to macrophages or their débris.

Another minor variation of the same picture was seen in rat 1313 (exposure 15 months and sacrificed without a holding period) in which the nodules were moderately cellular and collagenized. The nodules contained little silica, but the air spaces, which were filled with lipidic macrophages and their débris, contained a considerable amount of dispersed silica. Still another finding was noted in rat 1312 (exposure 15 months and sacrificed following a holding period of 9 months). Here there was a region of alveolar fibrosis associated with cholesterol crystal clefts. The latter were partially outlined by silica flocs (Fig. 17). Other silica flocs were found largely within air spaces related to macrophages or cell débris. Interstitial deposits were less abundant.

Severe reduction in mineral content of silicotic tissue was found in a pneumonic region of rat 1308 (silica exposure 15 months; death from intercurrent pneumonia 8 months later). Here the silicotic nodules were identified with difficulty because of the inflammatory changes (Fig. 19). Very little silica was found within the nodules and practically none was present elsewhere.

In a photomicrograph of medium magnification (rat 1315: sacrificed following a silica dust exposure of 12 months without a holding period), most of the alveolar macrophages appeared surrounded by a coating of silica which followed the cellular outline closely (Fig. 22). The fact that most cell bodies in the picture were dark suggested that relatively little silica was intracellular. Even where silica appeared to be within the cell, an extracellular position could not be excluded. Some of the cellular débris also appeared to lie in a slurry of finely divided silica. When the ash patterns of the individual macrophages were examined separately from the composite, it was noted that they were not ring-like structures. They were solid. Furthermore, they had their greatest density at the periphery (Fig. 21).

HUMAN SILICOSIS

The mineral pattern of different silicotic nodules from the 77-year-old patient who died of pulmonary embolism also presented various modifications. A small, perivascular nodule contained a moderate amount of silica, irregularly dispersed in coarse flocculent aggregates which were particularly dense in regions of anthracotic pigmentation (Fig. 24). A second, larger silicotic nodule had abundant silica in its center, whereas the acellular, hyaline, concentric periphery was practically free of silica (Fig. 26). Again the anthracotic pigment marked the sites of particularly heavy deposits of mineral. A third silicotic

nodule had no silica in its center but contained irregular concentrations of silica in the dense, collagenous periphery (Fig. 28). Finally, a larger, less well circumscribed silicotic nodule had an abundant silica content throughout its cross section (Fig. 30). However, there were radial, flame-like extensions of silica infiltration into adjoining interstitial tissues. The other nodules, previously mentioned, also were associated with some degree of direct extension of the silica by infiltration of the adjoining interstitial tissues. In addition, a few scattered fine flocs were found clinging to alveolar walls.

The pulmonary nodules in the second patient contained much more carbon and were considerably smaller and irregular in shape. Some of the nodules contained very little silica, whereas the mineral content of others was moderately heavy. Infiltration of nearby alveolar walls by silica was fairly common. The presence of silica flocs in the edema fluid within air spaces was of particular interest.

Discussion

In the earlier phases of this study, great difficulties were encountered while attempting to match some of the mineral patterns to the histologic pattern. Because in some instances there seemed to be no correlation between these two patterns, it was assumed that errors had been made in the location of the microscopic fields photographed. This difficulty is illustrative of the unexpected variability of the mineral content of silicotic lesions.

In a few of the photomicrographs it was not possible to be certain that the negatives had been properly matched because of the absence of landmarks. In most cases, however, the presence of multiple scattered points in one negative, some representing diameters of no more than 3 to 5 μ, and the presence of identical points in the other negative, facilitated the superimposition of the two images with an accuracy which probably was in many instances within a range of a few microns.

A silicotic nodule generally is believed to be produced by the irritation of the silica which is more or less embedded within the nodule. The presence of silica within a silicotic nodule was, therefore, an anticipated finding and its absence from such a nodule requires explanation. It is highly probable that at some time in the genesis and maturation of a silicotic nodule, it was completely or nearly completely filled with silica. If this assumption be correct, the demonstrated absence or near absence of this mineral from many of the sections of the silicotic nodules can be interpreted as a demineralization.

The concept of demineralization of silicotic nodules implies a lability

of the silica deposits. This lability or mobility of silicotic deposits is readily demonstrated by the presence of silica flocs free within alveolar spaces in animals which had been removed from exposure to dust for many months. Similar findings in aged men who presumably had not been exposed to silica dust for many years are also illustrative of the mobility of silicotic deposits. The mobility of other pneumoconiotic deposits has been reported previously from this laboratory.[1]

As a prerequisite to a proper consideration of the mechanism by which demineralization occurs, it is necessary first to develop a reasonable concept of the physical state of the mineral deposits.

Since it is impossible to predict from the stained section where silica deposits will be found within a nodule, it is apparent that such deposits are largely extracellular. This extracellular position is verified by photomicrographs of acellular, hyaline silicotic nodules with mineral deposits. By virtue of their extracellular position, the mineral particles are bathed in tissue fluid. In effect, the silica deposits are in the form of an aqueous suspension, a slurry, and as such the particles are capable of being transported with movements of the tissue fluid. This transport is, of course, modified by the filter-like impediment offered by the latticework of interstitial fibers. When edema supervenes, there is a reversal of the direction of flow of tissue fluid, the velocity and amount of flow are increased, and the interstices between the interstitial fibers become enlarged. All of these factors favor the transport of mineral in a direction opposite to that which caused its deposition.

An excellent insight into the fluid, paint-like character of intrapulmonary silica is seen in the halo-like covering of macrophages (Fig. 22) effected by the silica. The ash pattern of the individual "halo"-invested macrophage is suggestive of a hollow sphere since the pattern is in the form of a circular disk with a diameter greater than that of the associated macrophage and with a denser periphery.

The frequently noted location of mineral deposits in the peripheral portions of silicotic nodules and particularly the infiltration of near normal alveolar walls adjoining the nodules by silica flocs are thus explained by the interstitial movement of tissue fluid which acts as a vehicle for the particles. The escape of the mineral into the air spaces requires the assumption that defects exist in the respiratory membrane which allow for the escape of the slurry from the interstitium into the air space. These defects could be of the nature of erosions as postulated by this laboratory[2] or (what seems to be more plausible) caused by the retraction of cellular processes of the alveolar pneumocytes as postulated by Macklin[3] and by von Hayek.[4]

It is probable that the smooth-contoured, dense, homogeneous silica masses which completely fill a nodule represent the optimum silica content of such nodules. An increased fluid content of the nodule would tend to separate the aggregates of the mass and change its homogeneous character to a flocculent one. At the same time, the transport of some of the flocs from the periphery of the mass tends to replace its smooth contour by a serrated outline. It is significant that when silica flocs are found outside of the interstitium they are generally within those alveolar spaces which are in close proximity to partially demineralized silicotic nodules. This suggests that the intra-alveolar silica is derived from the nodules. Alveolar stasis as an alternate explanation is not readily acceptable in the case of animals which received a single intratracheal injection 12 months previously.

While much of the intra-alveolar silica is within lipidic macrophages, some is found associated with lipidic cellular débris. In addition, scattered flocs of silica are seen adherent to the free border of the alveolar wall. The lipidic state of the macrophages is probably related to their silica content. However, silica is not the only material which induces a fatty degeneration in macrophages, since it occurs also with antimony trioxide[5] and with silicates.[6]

Just as surprising as the demineralization of nodules is the presence of silica flocs within and upon alveolar walls which show no discernible reaction to their presence. Several explanations are possible. It may be that the particles had only recently arrived at the scene and that sufficient time had not yet elapsed for a reactive process to develop. On the other hand it may be—and to us this seems more likely—that the reactive fibrosis typical of silicosis is caused by the lipids secondary to silica rather than by silica itself (Fallon's theory).[7] This theory allows for a somewhat longer interval between the arrival of silica particles to the interstitium and the beginning of fibrosis, since first there must be phagocytosis, followed by lipidic degeneration, death, and disintegration of the phagocyte. A third explanation could be that the shifting about of silica particles from one place in the interstitium to another, and from one portion of the respiratory membrane to another, has been too rapid to allow for damage, and hence, for reaction to occur.

The most extreme examples of demineralization probably occur in pneumonic regions. Here the reduction in the silica content of nodules and elsewhere has been so severe that frequent difficulties have arisen in attempting to match the few scattered particles visible in the ash pattern to the densely cellular histologic pattern.

It has been a repeated observation that silica flocs tend to concentrate in the subpleural interstitium and air spaces as well as within the pleura itself. It is recognized that a portion of the pulmonary lymph flow is directed toward the pleura. There is also probably a parallel flow of tissue fluid in the same direction. This would account for a subpleural interstitial concentration of silica. To explain a subpleural alveolar concentration of silica (Fig. 9) it is necessary to assume the occurrence of multiple break-throughs, allowing interstitial fluid to pour through the respiratory membrane and to sweep the suspended mineral into the air spaces.

The interstitial redistribution of silica which is a result of the demineralization of silicotic nodules could serve as an explanation for the progressiveness of silicosis. This implies that the silica particles have not lost their pathogenicity by their prolonged contact with tissue fluid. However, it should be recalled that redistribution of other pneumoconiotic deposits also occurs[1] without rendering such pneumoconioses progressive. The difference, of course, lies in the primary pathogenicity of the silica particle. Theoretically, the transport of silica from the interstitium back into the air spaces by tissue, or edema fluid should favor the removal of this mineral from the lung via the bronchi. Although such removal undoubtedly occurs, its effectiveness apparently is not sufficient to render the disease non-progressive.

The silica content of lung tissue, when considered as a percentage of the dry lung weight, has been an uncertain criterion of the presence of silicosis, although a better parallelism has been claimed for the total weight of silica found in the lung.[8] The findings described in this paper seem to offer a reasonable explanation for the discrepancies between chemical analysis and anatomical findings in silicosis.

Summary

A photographic method for relating insoluble mineral deposits in the lung to histologic structures has been described. The method has been applied to the study of experimental silicosis and several cases of human silicosis. Experimental silicosis was produced by the intratracheal injection of silica and also by the inhalation chamber technique.

The amount and distribution of mineral in silicotic nodules were found to be highly variable in the experimental animal as well as in the two human cases studied, ranging from abundance to near absence.

The near absence of demonstrable mineral from silicotic nodules is interpreted as due to demineralization. This demineralization is a

manifestation of the lability or mobility of intrapulmonary silica deposits. Other manifestations of this mobility are found in the scattered interstitial extensions of silica deposits peripheral to silicotic nodules as well as in the presence of silica flocs within air spaces situated in proximity to partially demineralized nodules. The causes of demineralization are edema and inflammation In fact, the most clear-cut examples of demineralization are seen in pneumonic regions.

Silica flocs are demonstrable within and upon alveolar walls which show no significant changes in structure. Aggregates of silica are also found in edema fluid.

The demineralization of silicotic nodules and the subsequent redistribution of silica particles to unaffected portions of the lung offer an attractive explanation for the progressiveness of silicosis.

The demineralization of silicotic nodules in association with a removal of liberated silica via the tracheobronchial tree also offers an explanation for the unreliability of the silica content of the lung as a criterion of silicosis.

We acknowledge with gratitude the constructive criticism given by Dr. Howard T. Karsner. Thanks are due also to Miss Ethel B. Tolker for her meticulous care in the preparation of slides.

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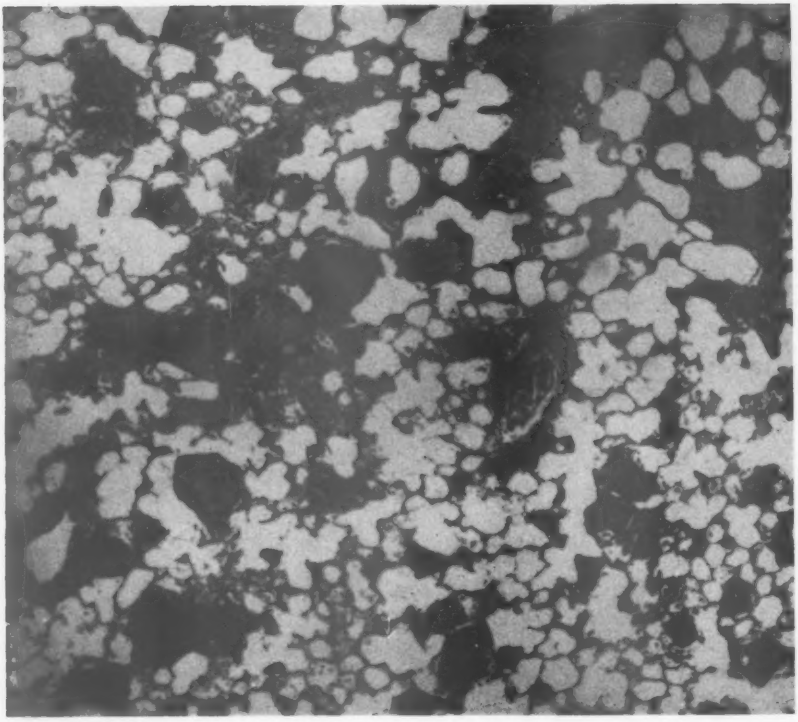
[*Illustrations follow*]



1

LEGENDS FOR FIGURES

FIG. 1. Section of lung from rat 1749, sacrificed 2 months following an intratracheal injection of 40 mg. of silica. The silicotic nodules at this stage are cellular and contain little or no collagen. Some alveoli contain scattered macrophages. Hematoxylin and eosin stain. × 53.

FIG. 2. Silica ash pattern of the field shown in Figure 1. × 49.

FIG. 3. Composite print by superimposing silica ash pattern upon Figure 1. The silica masses are generally dense and occupy all of the nodule except for a narrow periphery. Some aggregates are composed of separated flocculent masses. The intervening parenchyma contains many silica flocs adherent to alveolar walls and within macrophages. × 49.

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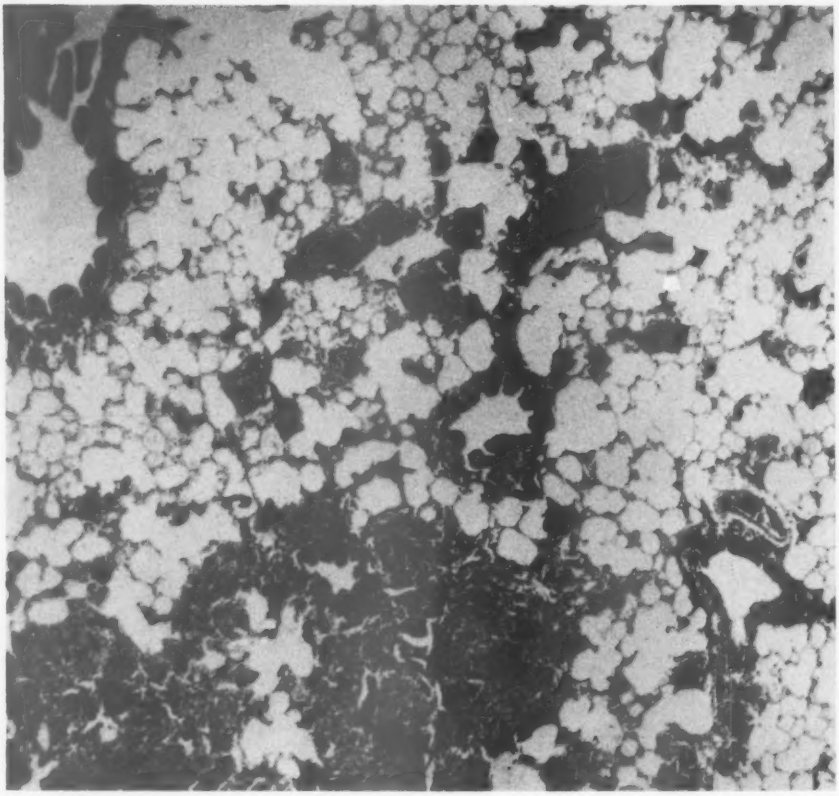

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FIG. 4. Another region of lung from rat 1749. In addition to small nodules, there are larger nodules which form large solid masses by confluence. Some alveolar macrophages are present. Hematoxylin and eosin stain. × 53.

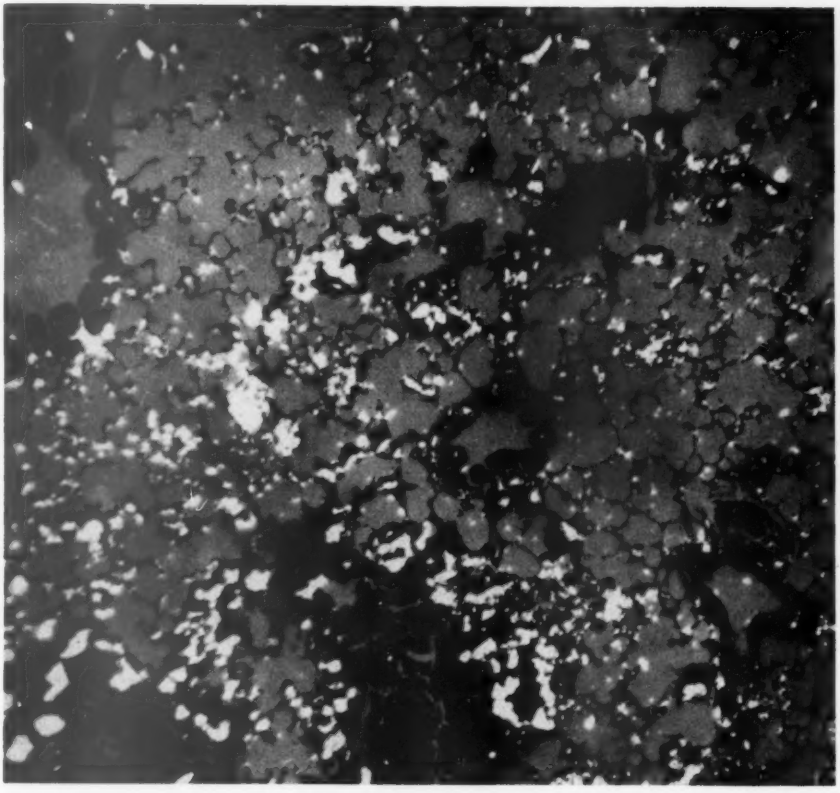

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FIG. 5. Composite print of the area of Figure 4, showing abundant silica within alveolar macrophages, free within air spaces, and clinging to alveolar walls. In contrast, most of the nodules are largely devoid of silica. × 53.

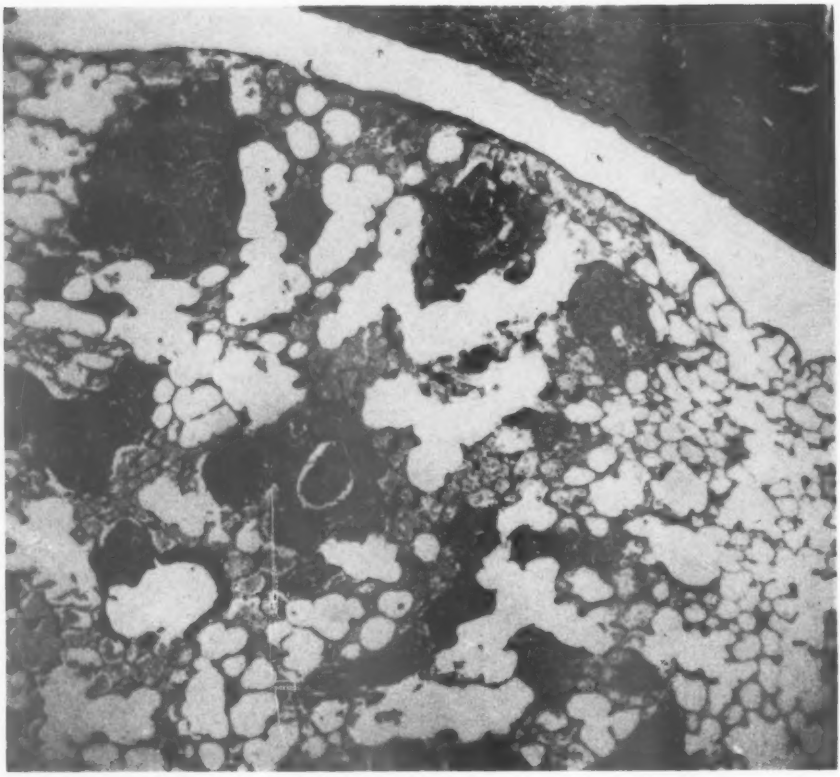

FIG. 6. Rat 1750, similar dosage as the preceding, and also sacrificed at 2 months. Nodules of the same type as in the preceding figures. Many air spaces adjoining the nodules are filled with foam cells. In one corner is a portion of an atelectatic lobe. Hematoxylin and eosin stain. × 53.

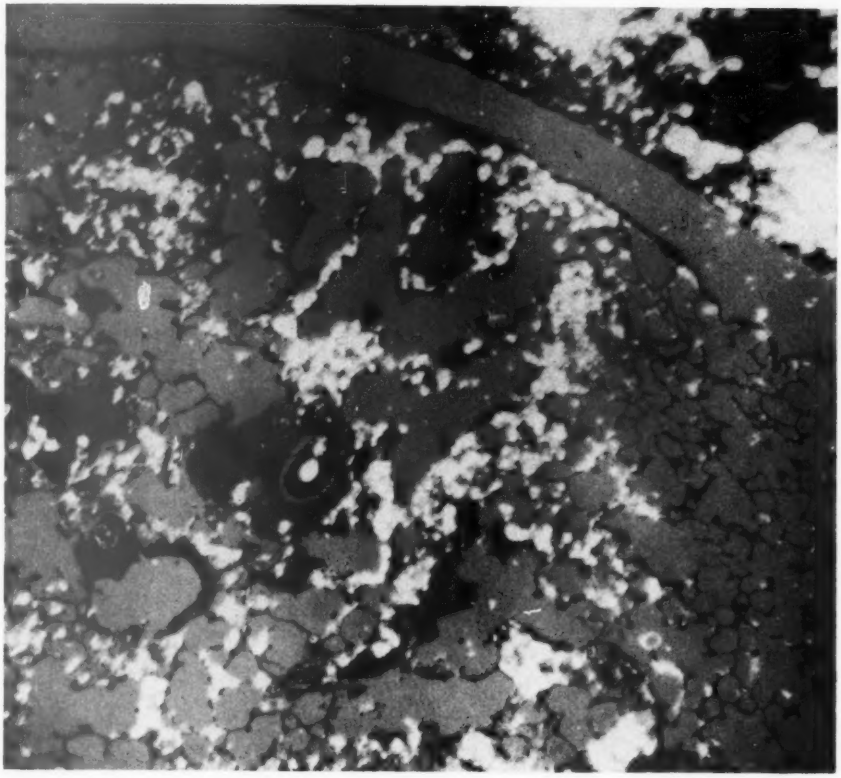

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FIG. 7. Composite print of the area of Figure 6, showing most of the nodules devoid of silica except for relatively few flocculent aggregates. Instead, there is abundant silica within the alveolar lipidic macrophages. Very little extracellular silica is seen. × 53.

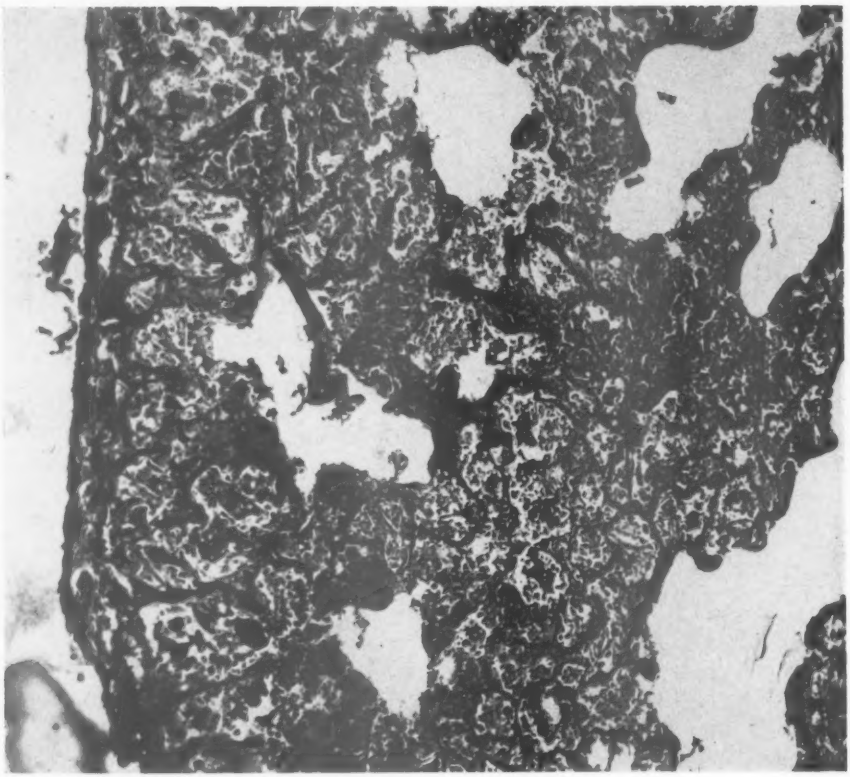


Fig. 8. Rat 1753. 40 mg. of silica intratracheally. Death from intercurrent pneumonia 5 months later. Subpleural region of diffuse alveolar fibrosis associated with macrophages and cellular débris in air spaces. Periodic acid-Schiff's (PAS) stain. × 113.

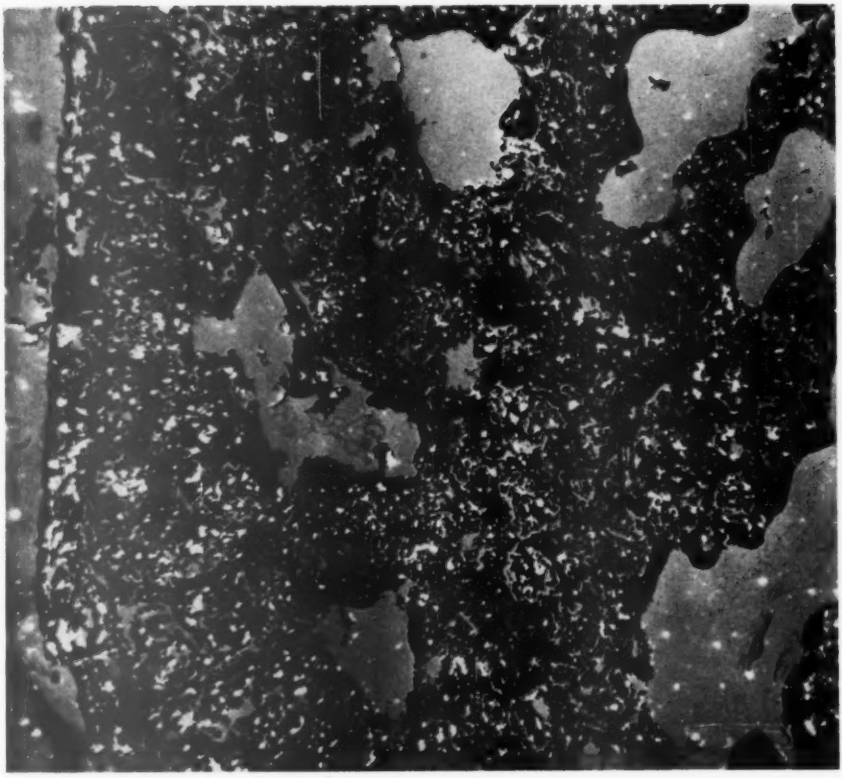
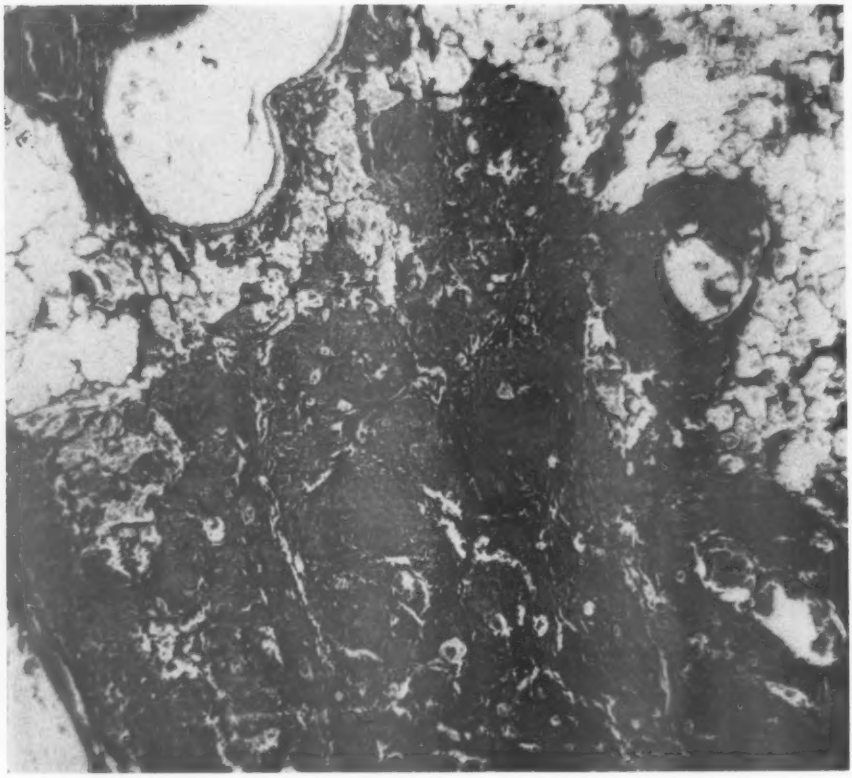
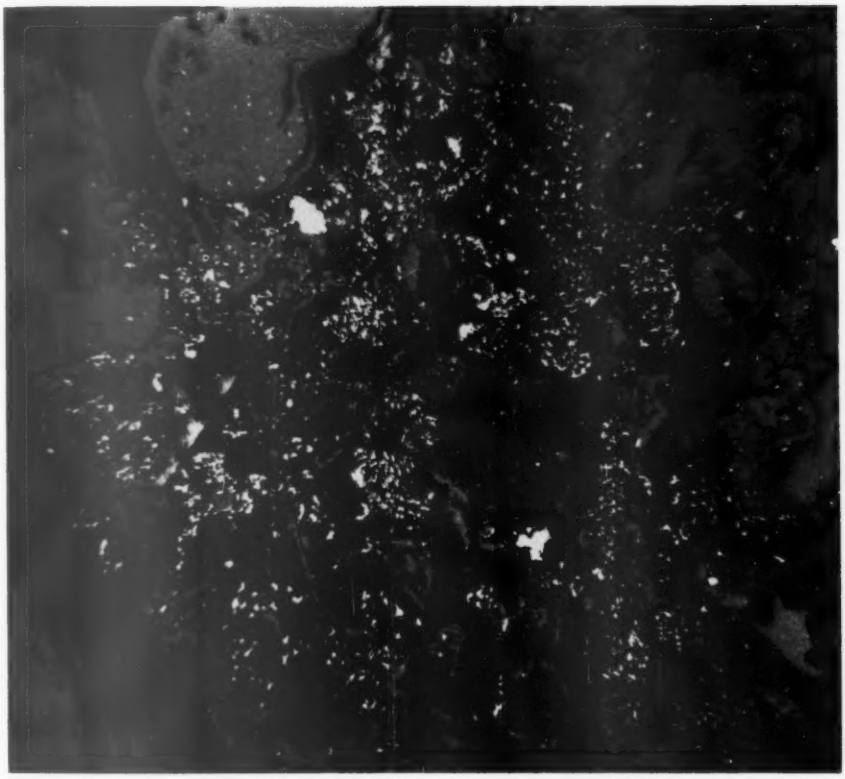


FIG. 9. Composite print of the area of Figure 8, showing diffuse infiltration of alveolar content by silica, generally finely divided but also in coarser flocs, particularly near the pleura. The interstitial tissues also are infiltrated by fine and coarser silica flocs. × 113.



10

FIG. 10. Rat 1759, 40 mg. of silica intratracheally. Sacrificed 12 months later. Clusters of confluent silicotic nodules which are well collagenized. There is focal emphysema. Hematoxylin and eosin stain. × 35.



11

FIG. 11. Composite print of the area of Figure 10 shows relatively little silica within the nodules. A number of the nodules are entirely free of silica while some of the air spaces contain an appreciable amount, apparently within alveolar macrophages. × 35.

12

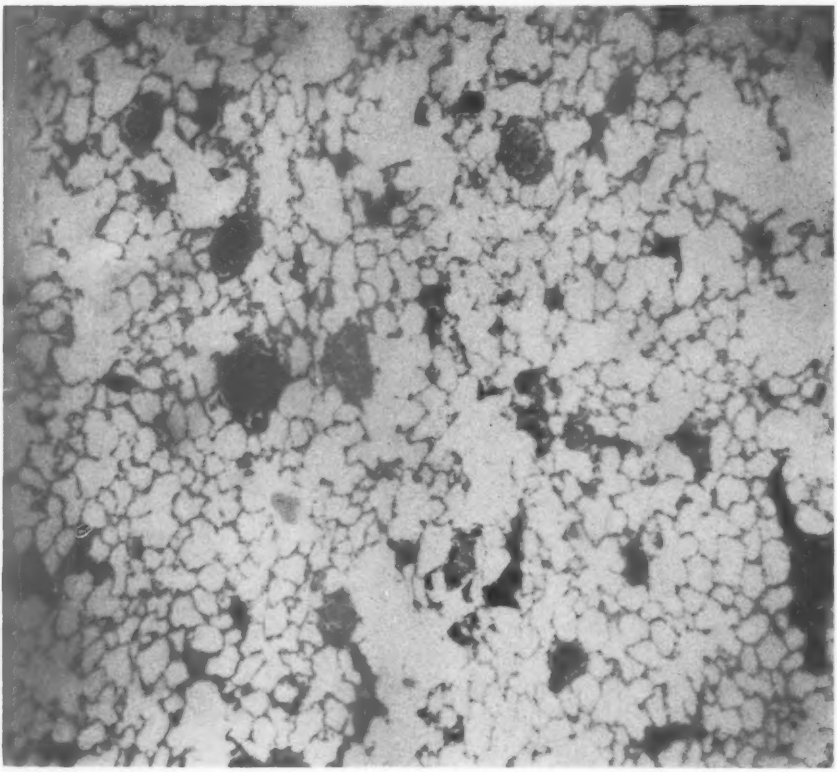
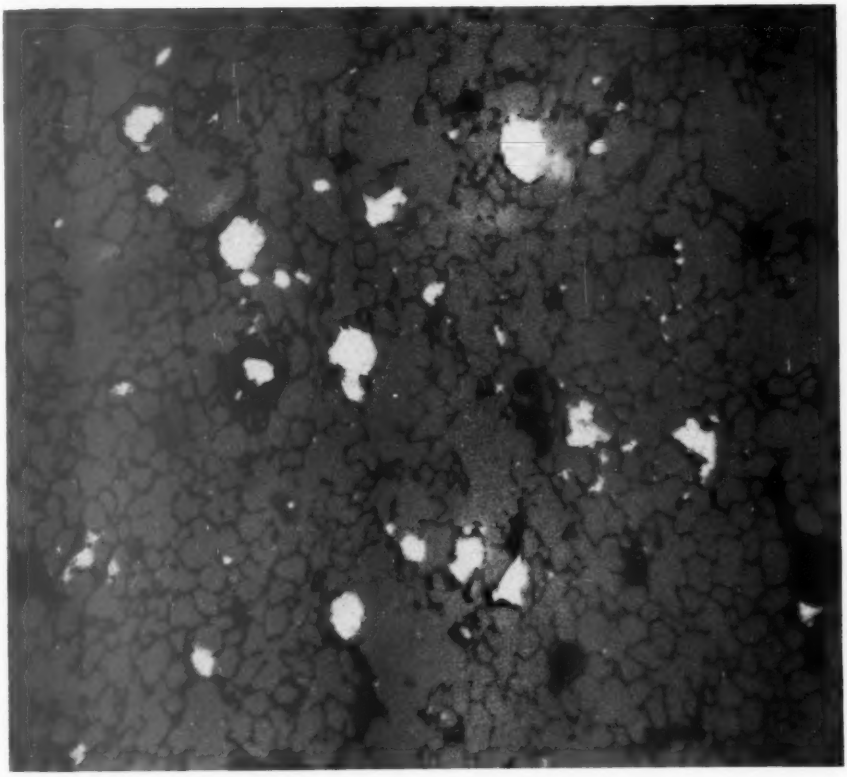


FIG. 12. Rat 1363 exposed to silica dust in an inhalation chamber for 15 months and sacrificed after a holding period of 9 months. There are discrete silicotic nodules which are cellular but are also moderately collagenized. The intervening parenchyma is generally near normal. Hematoxylin and eosin stain. × 35.



13

FIG. 13. Composite print of the area of Figure 12, showing homogeneously dense silica masses, in many instances nearly completely filling the nodule. Portions of the outlines of the silica masses are smooth and sharp; other portions are fuzzy. There are only a few scattered small silica flocs outside of the nodules. × 35.

14

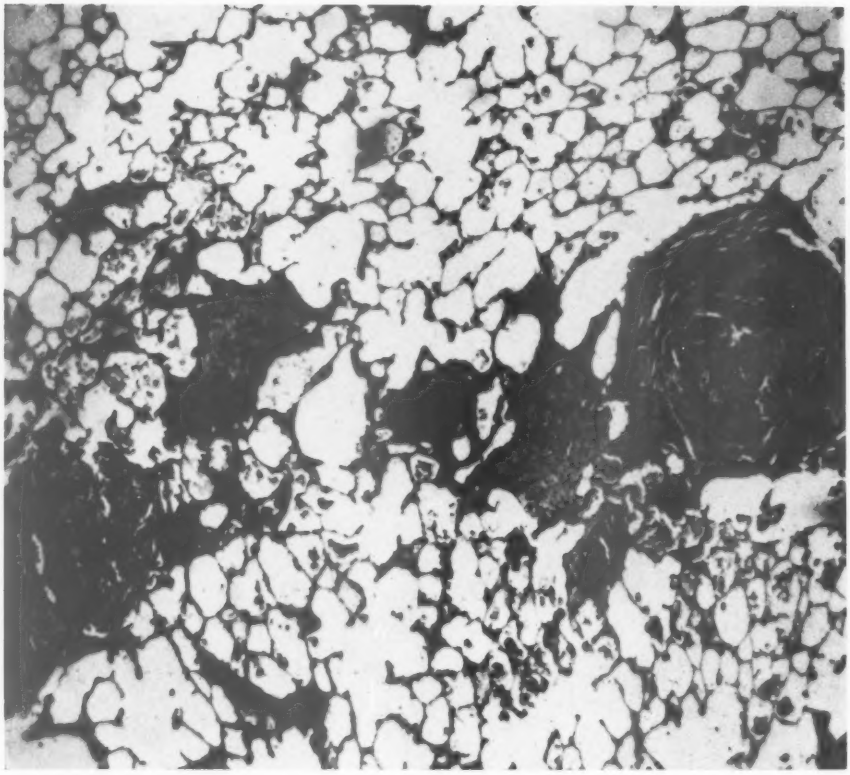


FIG. 14. Rat 1338 exposed to silica dust in an inhalation chamber for 15 months and sacrificed after a holding period of 7 months. There are two well collagenized, more or less rounded nodules, adjacent to which are several secondary "younger" nodules. Many air spaces in the vicinity of the nodules contain alveolar macrophages. Focal alveolar fibrosis also is present. Hematoxylin and eosin stain. × 53.

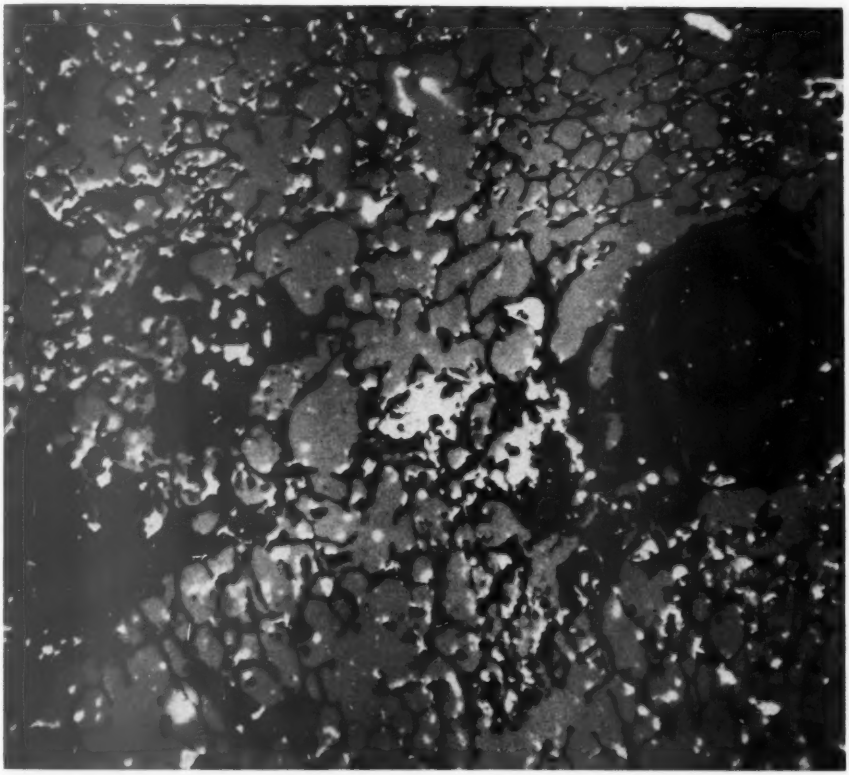


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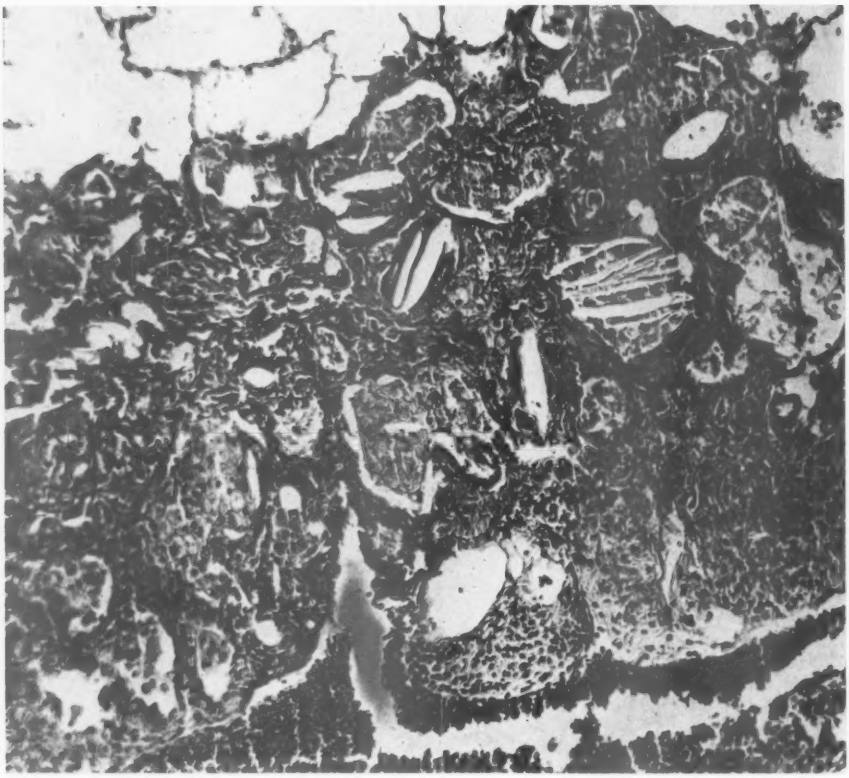
FIG. 15. Composite print of the area of Figure 14, showing almost complete absence of silica from well collagenized nodules and more aggregations of silica flocs in the "younger" nodules. There is abundant silica within the air spaces and adherent to alveolar walls. × 53.



16

FIG. 16. Rat 1312 exposed to silica dust in an inhalation chamber for 15 months and sacrificed after a holding period of 9 months. A region of diffuse alveolar fibrosis associated with cholesterol-crystal clefts. The air spaces contain débris and macrophages. Hematoxylin and eosin stain. × 113.

17

FIG. 17. Composite print of the area of Figure 16 shows much silica within the macrophages and débris in the air spaces. Several of the cholesterol-crystal clefts are partially outlined by silica flocs. Some silica also is present within interstitial tissue. × 113.

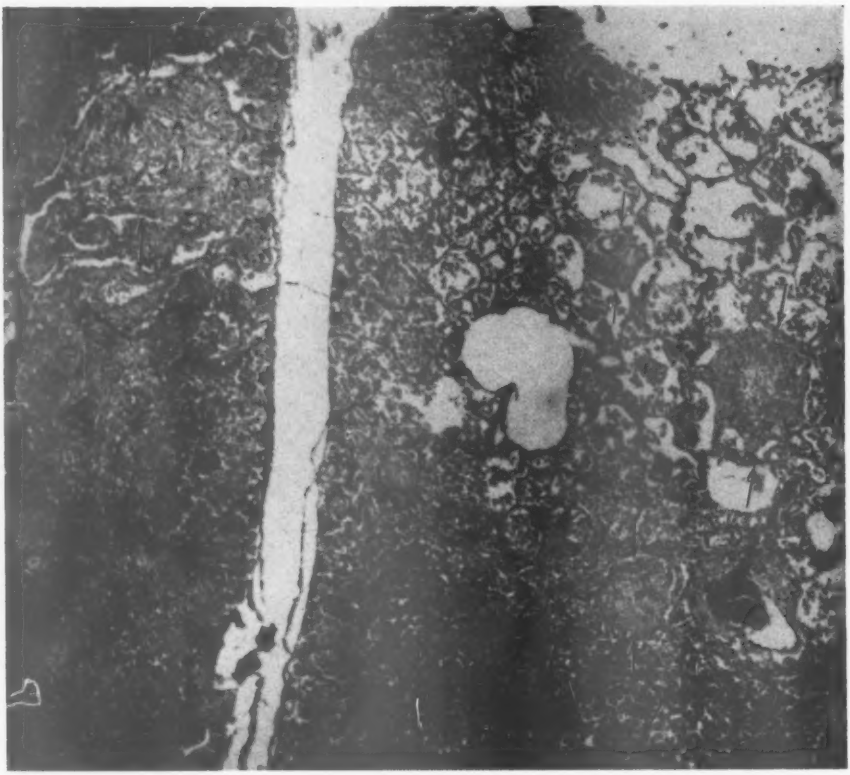


FIG. 18. Rat 1308 exposed to silica dust in an inhalation chamber for 15 months. Death from intercurrent pneumonia 8 months later. There is extensive pneumonia which all but obscures the silicotic nodules (arrows). Hematoxylin and eosin stain. × 53.

19

FIG. 19. Composite print of the area of Figure 18 shows very little silica remaining in the nodules and there is practically none in the air spaces. The pleura, however, is outlined by a fine deposition of silica. × 53.

20

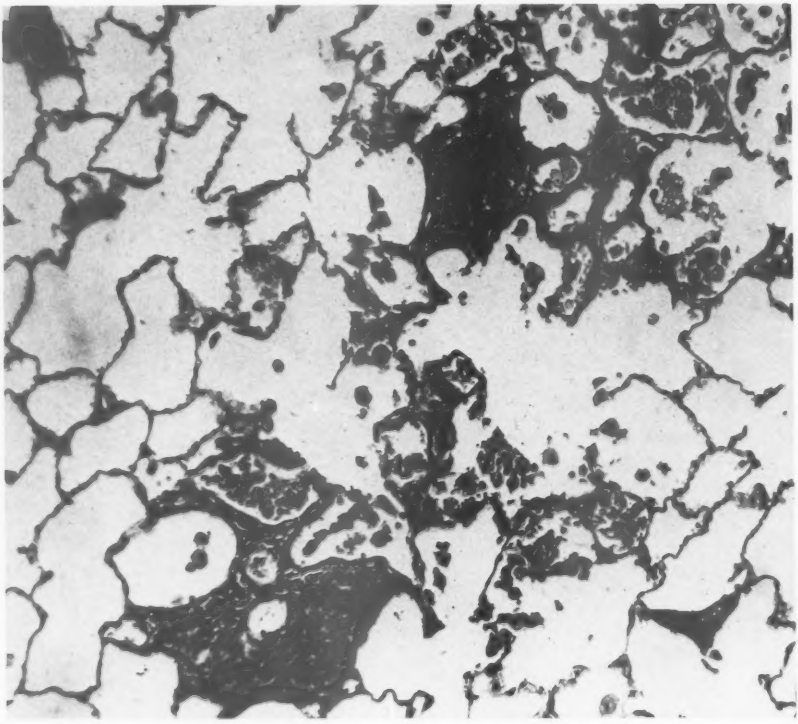
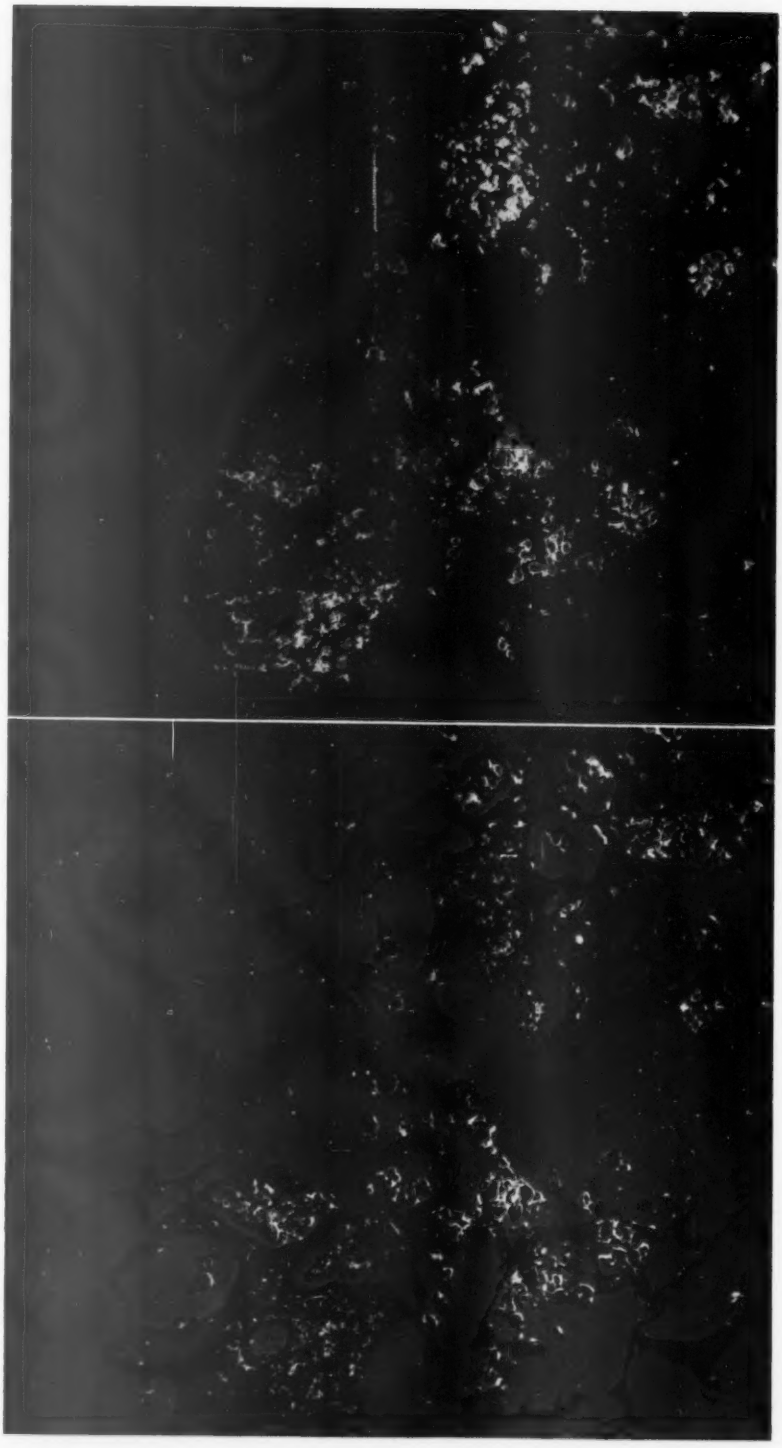


FIG. 20. Rat 1315 sacrificed following 12 months of exposure to silica dust in an inhalation chamber. There are foci of interstitial and alveolar fibrosis associated with the presence of macrophages and cellular débris within alveolar spaces. Hematoxylin and eosin stain. × 105.

FIG. 21. Acid-washed ash pattern. Many of the rounded structures represent the silica associated with a single macrophage. The low density in the center of the structures and their greater density at the periphery are of significance. This appearance suggests that the silica coats the outside of the macrophage. × 105.

FIG. 22. Composite print, combining Figures 20 and 21, shows a moderate amount of silica within the foci of interstitial and alveolar fibrosis. More silica is found within the air spaces associated with the macrophages and débris. It is interesting to observe that much of the silica is extracellular, frequently covering alveolar macrophages with a moderately thick coating. A small amount of silica is adherent to alveolar walls. × 105.



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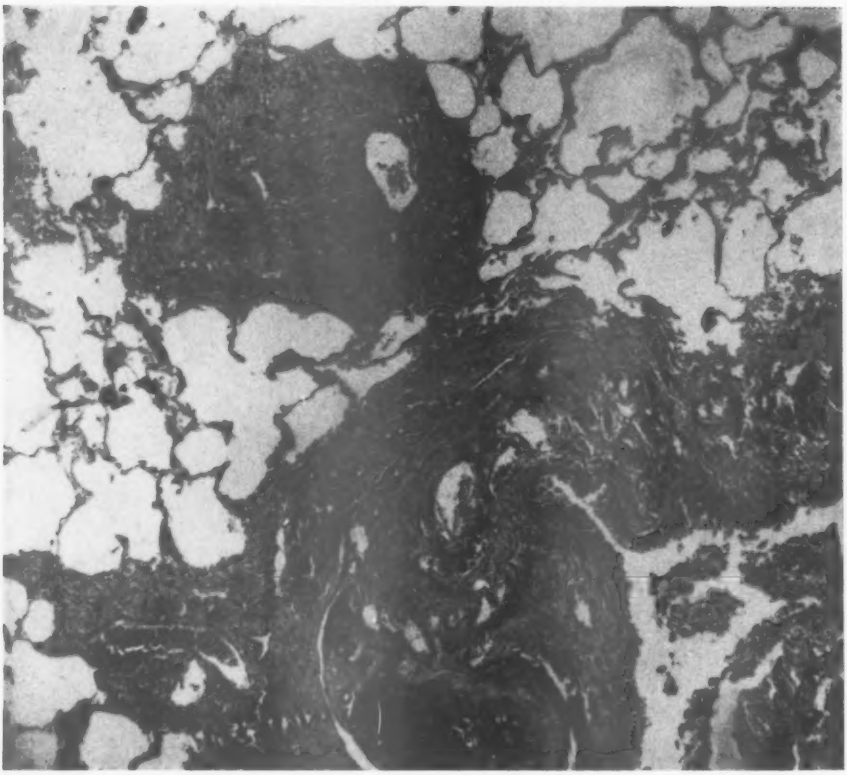
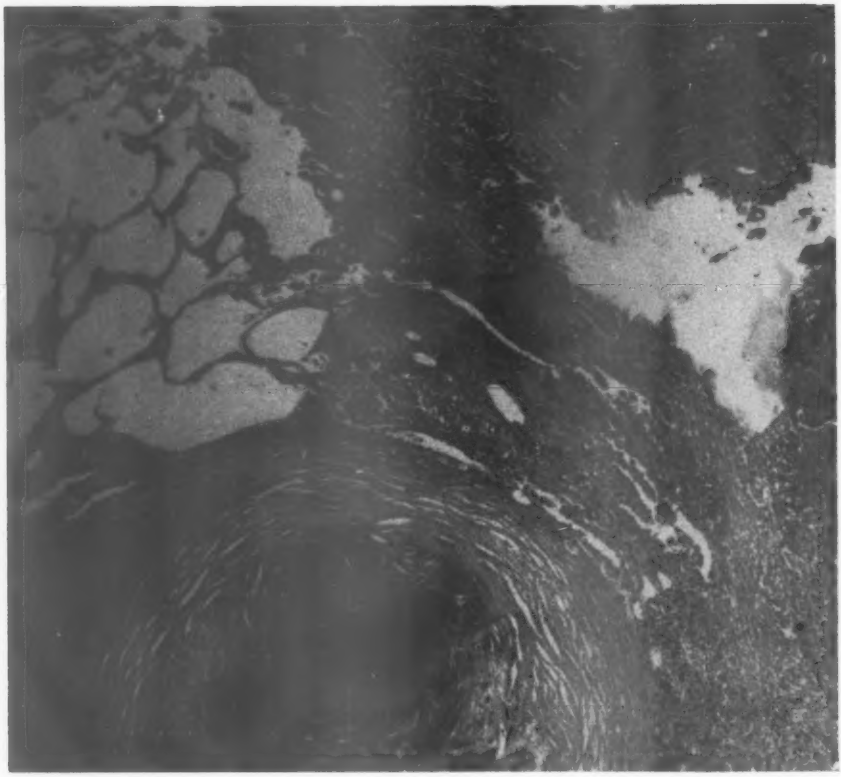


FIG. 23. A small perivascular, immature nodule is situated adjacent to a bronchus. A scattering of anthracotic pigment is found in the peripheral portion of the nodule. This section is from a white man, 77 years old, who died of pulmonary embolism. Anthracosilicosis was asymptomatic and an incidental finding. Hematoxylin and eosin stain. × 53.

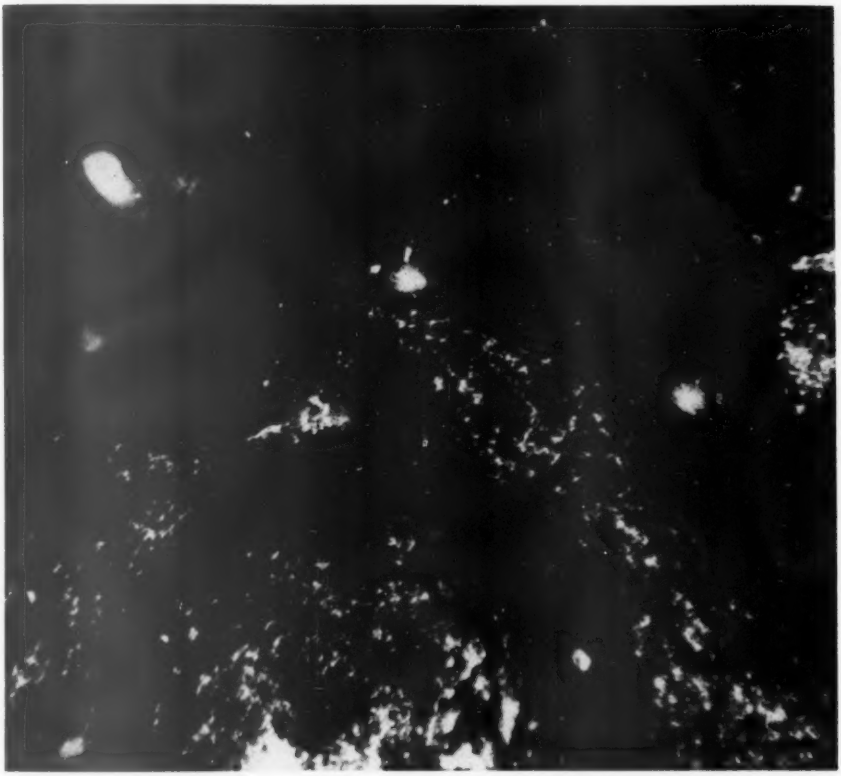
24

FIG. 24. Composite print of the area of Figure 23, showing the presence of a moderate amount of silica within the nodule. The silica is not uniformly distributed, being particularly dense in the regions of anthracotic pigmentation. Small silica flocs, isolated or clustered, are found within adjacent alveolar walls and in the air spaces, generally clinging to the alveolar walls. × 53.



25

FIG. 25. A large silicotic nodule from the same patient as that from whom Figure 23 was derived. The whorled center is dark because the section is excessively thick there. Adjacent to the concentric, hyaline, acellular periphery there is compressed, atelectatic parenchyma infiltrated by anthracotic pigment. Hematoxylin and eosin stain. × 53.



26

FIG. 26. Composite print of the area of Figure 25 shows abundant but irregularly disposed silica in the center of the nodule. The acellular, hyaline, concentric capsule is practically devoid of mineral while a considerable amount of well dispersed silica is found throughout the adjoining atelectatic parenchyma. × 53.

27

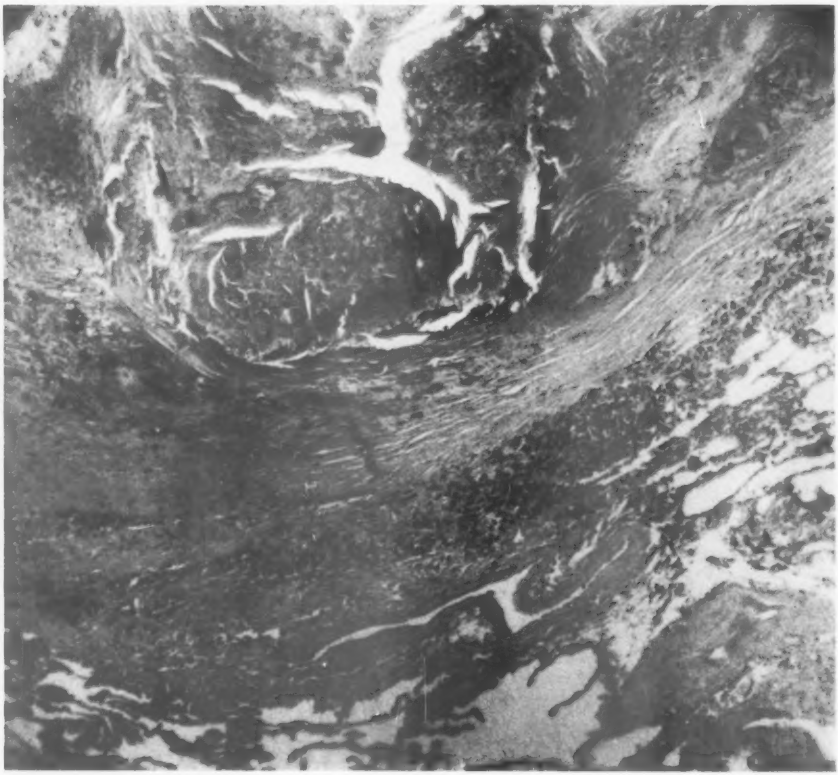
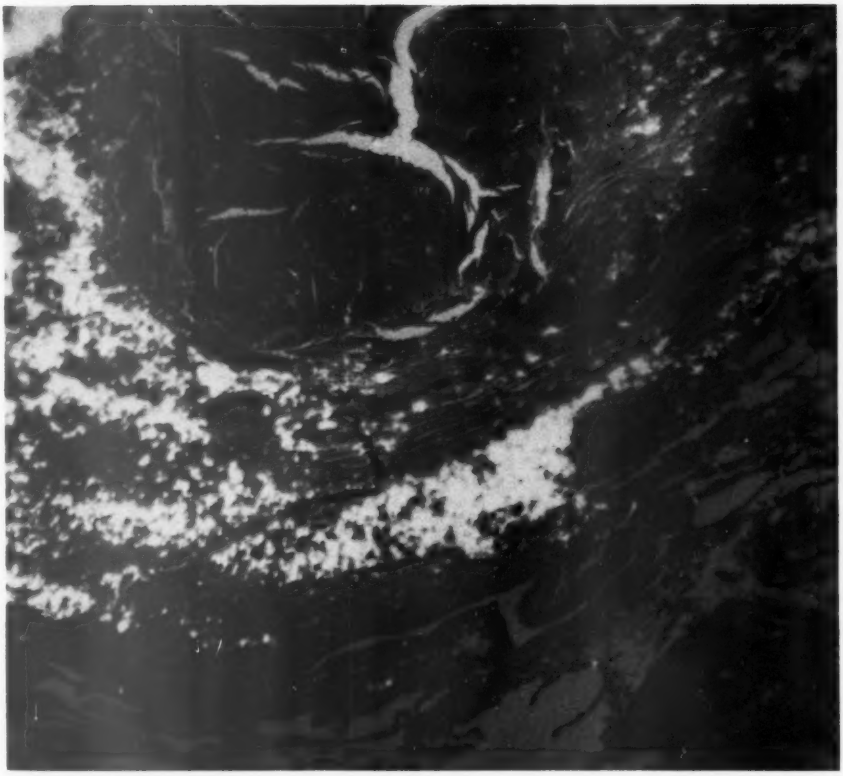


FIG. 27. A portion of a silicotic nodule from the same patient as that from whom Figure 23 was derived, showing a necrotic center which has artefactitious cracks due to shrinkage. Adjoining the concentrically arranged, hyaline, collagenous periphery there is anthracotic, fibrotic, and atelectatic alveolar tissue. Hematoxylin and eosin stain. × 53.



28

FIG. 28. Composite print of the area of Figure 27, showing complete absence of silica from the center of the nodule and focal absence of the mineral from the hyaline peripheral tissue. Silicotic deposits are heavy in regions of anthracotic pigmentation but are not limited to these foci. × 53.

29

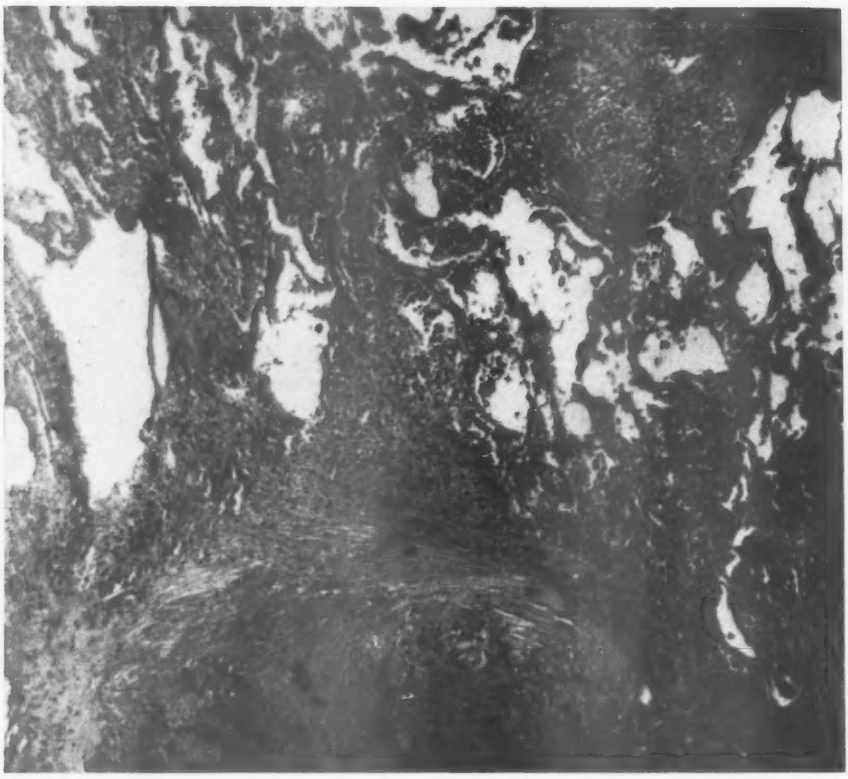
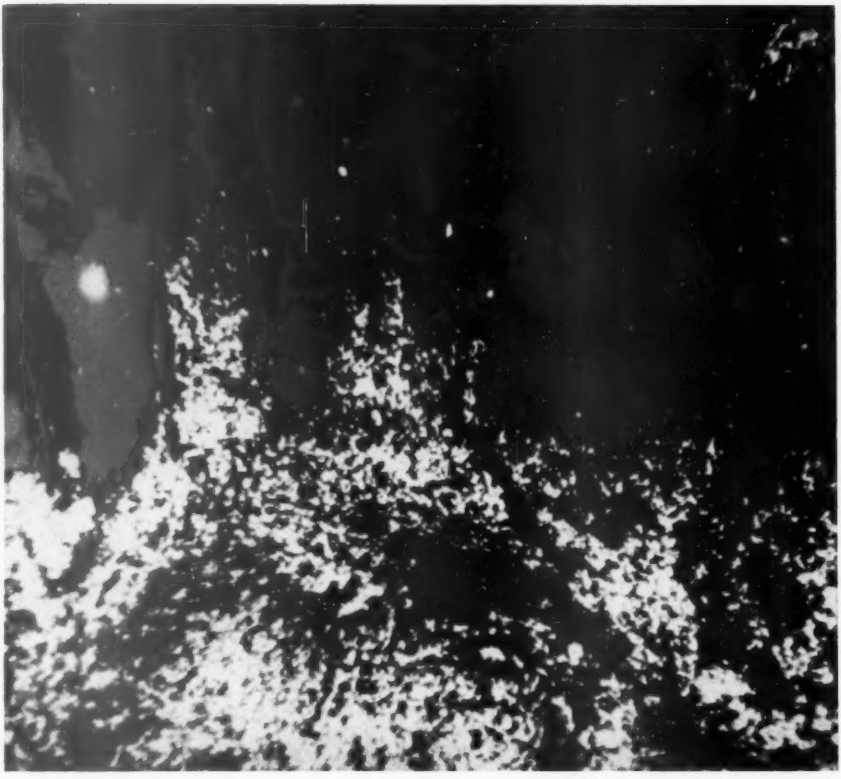


FIG. 29. A portion of a silicotic nodule from the same patient as that from whom Figure 23 was derived, showing whorled, acellular, collagenous tissue bordered by more cellular and somewhat anthracotic stroma. Thickened alveolar walls adjoin the periphery of the nodule. Hematoxylin and eosin stain. × 53.



30

FIG. 30. Composite print of the area of Figure 29, showing abundant silica with heavy flame-like extensions of silica flocs into adjoining alveolar walls. × 53.

VISCERAL LARVA MIGRANS

WITH A CASE REPORT *

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From the Departments of Pathology (Drs. Dent and Carrera) and of Tropical Medicine and Public Health (Drs. Nichols and Beaver), Tulane University School of Medicine, and the Department of Pathology, Southern Baptist Hospital (Dr. Staggers), New Orleans, La.

Visceral larva migrans[1] is the term applied to a clinical syndrome resulting from the invasion of human viscera by the larvae of nematodes normally parasitic in lower animals. This syndrome occurs primarily in children and is characterized by a pronounced and prolonged eosinophilia with or without constitutional symptoms.[2] Prior to the demonstration of human visceral invasion by this group of nematodes, the syndrome was reported as Loeffler's syndrome, tropical eosinophilia, familial eosinophilia, benign eosinophilic leukemia, or disseminated visceral ascariasis wth eosinophilia. Thus far, only the dog and cat ascarids of the genus Toxocara have been identified as responsible for this syndrome.[3-6] Although the larvae of these parasites are not adapted to complete their life cycle within the human, they are capable of invading human viscera in which they may survive for many months.

In structure, the larvae of Toxocara resemble those of the more familiar *Ascaris lumbricoides*, and in their normal hosts—the dog and cat—the larvae follow a life cycle similar to that of *A. lumbricoides* in man. Nichols[7,8] described the morphology of the Toxocara larvae, differentiating them from closely allied species. He pointed out that the shrinkage in length of the larvae in preparation of tissue specimens may amount to one fifth of the original length. *Toxocara canis* generally has a maximum diameter range of 18 to 21 μ while *Toxocara cati* is about 3 μ smaller. The reconstructed larvae of both species had an average length of 320 μ. Nichols further showed that it was not possible to differentiate *T. canis* from *T. cati* in tissue sections except on the basis of the over-all dimensions. He emphasized the difficulty in making a species diagnosis from the structural characteristics of larvae encountered in biopsy or necropsy material, since other species of larvae having a similar structure might be responsible for granulomas in human viscera.

* This investigation was supported in part by a donation of the Children's Clinic, New Orleans, La., and in part by Research Grant B 752 from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service.

Received for publication, September 10, 1955.

Children acquire infection by swallowing dirt contaminated with infective ova derived from dog and cat feces. Headlee[9] and Beaver[10] have found the surface soil of dooryards, where the animals were confined, highly contaminated with infective ova. These ova remain viable for many months, even under adverse weather conditions. Frequently, they are found together with the ova of *A. lumbricoides*, and consequently, mixed infections in the human are probably common.

After the ova of Toxocara are swallowed, they hatch in the stomach and intestine, and the larvae migrate through the intestinal wall to the other organs, probably by way of blood vessels, lymphatics, and tissue spaces.

It is important to emphasize that while infection with *A. lumbricoides* can be diagnosed by parasitologic examination of the feces for the ova or adult worm, the larvae of Toxocara do not commonly complete their life cycle in man, and rarely, if ever, reach the egg-laying stage in the human intestine. Therefore, fecal examinations are noncontributory to the diagnosis of visceral larva migrans.

We have studied at necropsy the widespread lesions caused by the larvae of *T. canis* in a child who died from an incidental but overwhelming homologous serum hepatitis. The purpose of the present report is to describe the clinical and the pathologic findings.

Report of Case

G. J. P., a white male, 19 months of age, was first seen by us on November 11, 1952, with fever, abdominal distention, and general malaise. The patient had been hospitalized elsewhere for 6 weeks, acutely ill with fever, a hacking non-productive cough, and abdominal distention. Shortly after the acute onset, the liver became enlarged and was palpated 6 cm. below the costal margin.

Laboratory findings during this time showed a total leukocyte count varying from 40,000 to 63,000 per cmm. with eosinophils ranging from 40 to 60 per cent of the total. The total red blood cell count and hemoglobin remained within normal limits. Agglutination tests for typhoid fever, paratyphoid fever, and brucellosis were all negative. Chemical examinations of the blood gave normal findings. Urinalyses and urine cultures were negative. Blood cultures were sterile. Spinal fluid examination revealed normal pressure and cell count. Numerous stool examinations were negative for ova, cysts, and parasites.

Six days of intensive antibiotic therapy, including penicillin, streptomycin, terramycin, and aureomycin, failed to ameliorate the symptoms. Subsequently, the patient received 100 mg. of hetrazan (diethylcarbamazine) three times daily for 10 days without improvement and with no diminution in the eosinophilic leukocytosis. During the first 3 weeks the patient was given three transfusions totaling 300 cc. of whole blood. A review of systems during this period failed to elicit any additional symptoms except irritability. No jaundice and no bleeding tendency was noted.

Prior to this illness, the patient had developed normally and had had none of the so-called childhood diseases. Routine immunizations against diphtheria, pertussis, and tetanus had been given. Inquiry revealed that he was a "dirt-eater." The family

owned several dogs that were confined to the dooryard in which the children played. There was no significant family history; both his parents, a brother, aged 4, and a sister, aged 3, were well. Examination of the siblings revealed the sister's white blood cell count to be 16,000 per cmm. with 20 per cent eosinophils. Subsequent examinations, extending over a period of 6 months, of this asymptomatic sibling revealed consistent eosinophilia of from 15 to 26 per cent. On the first stool examination a single ovum of *Trichocephalus trichiurus* was found; numerous subsequent stool examinations were negative for ova, cysts, and parasites.

The patient was admitted to the Southern Baptist Hospital in New Orleans on November 15, 1952. Physical examination revealed a well developed, well nourished white boy who weighed 28 lbs, 12 ozs. and was 32 inches in height. He was irritable but not in acute distress. The liver extended 6 cm. below the costal margin. There was no lymphadenopathy and no jaundice. The eye grounds were normal. There were no other significant findings.

Blood studies at this time indicated a hemoglobin content of 9.3 gm. per cent; erythrocytes, 3,401,000 per cmm.; white blood cells, 32,000 per cmm.; platelets, 247,000 per cmm.; reticulocytes, 6.8 per cent; and a hematocrit reading of 31. A differential study showed 28 segmented neutrophil cells, 2 band cells, 1 metamyelocyte, 59 eosinophils, 7 lymphocytes, and 3 monocytes. The Ivy bleeding time was 4 minutes; the Lee-White clotting time was 12 minutes. The erythrocyte sedimentation rate (Westergren) was 125 mm. in the first hour. Studies of the bone marrow revealed a striking increase in eosinophils at all stages of maturation. Roentgenograms of the long bones and skull were normal. Radiologic examination revealed diffuse pneumonitis in the lower half of the right lung field. The blood culture was negative. The cephalin-cholesterol flocculation test was 1 plus at 48 hours. The indirect bilirubin was 0.1 mg. per cent. Agglutination tests for typhoid fever, paratyphoid fever, and brucellosis were negative. The heterophile antibody titer was 1:7168. The heterophile absorption test was positive at 1:224. Kline, Wassermann, and Kahn tests were negative. The urine showed a slight trace of albumin and no bile or urobilinogen. Daily fecal examinations revealed no ova, cysts, or parasites. The Mantoux test for tuberculosis was negative at 1:100.

On November 19, approximately 7 weeks after the onset of illness, a laparotomy was performed and a segment of the liver, which measured approximately 3 cm. in diameter, was removed for histologic study. Grossly, the enlarged liver showed numerous white nodules varying from 1 to 3 mm. in diameter. These were visible through the capsule and were scattered throughout both lobes. Exploration of the immediate subhepatic area revealed no abnormalities.

Liver Biopsy. The hepatic cells were well preserved in the portion of liver obtained for biopsy. The architecture was normal except for numerous focal granulomas. Microscopically, these granulomas (Fig. 5) presented a central area of necrosis, palisaded epithelioid cells, and a dense infiltrate of eosinophils, neutrophils, lymphocytes, and plasma cells. Serial sections revealed the larvae of *T. canis* in many of them.

Preoperatively, 200 cc. of whole blood was given. The postoperative course was uneventful. The temperature was normal on the fourth postoperative day and remained normal until the patient was discharged. During the postoperative period there was marked general improvement. The laparotomy wound healed rapidly.

From November 20 until November 22 the daily white blood cell count varied from 36,000 to 40,000 white cells per cmm., with the eosinophils ranging from 45 to 54 per cent. During this period the hemoglobin was 11 gm. per cent and the red blood cells ranged from 4.4 to 5 millions per cmm. Morphologic studies of the peripheral

blood revealed most of the eosinophils to be mature but a few eosinophilic myelocytes were present. On November 22 the patient was started on multiple vitamins, hetrazan, and terramycin. On the same day, cortisone was begun orally in doses of 150 mg. daily.

On November 24 the hemoglobin level was 10.5 gm. per cent; erythrocytes, 4,100,000; blood platelets, 214,490 per cmm.; white blood cells, 12,050 per cmm. with 72 per cent neutrophils, 20 per cent lymphocytes, 4 per cent monocytes, and 4 per cent eosinophils. During the remainder of the hospital stay the blood count remained essentially unchanged. Cultures of the liver tissue taken for biopsy were negative. On the patient's discharge, November 27, the parents were advised to continue the medication at home, to isolate the patient from his dogs, and to prevent him from consuming dirt.

On December 30, 1952, the patient appeared greatly improved and was active and happy. He had gained 1½ lbs. The liver and spleen had diminished in size. The hemoglobin was found to be 11 gm. per cent; erythrocytes, 4,280,000 per cmm.; white blood cells, 20,000 per cmm. with 32 per cent eosinophils. Cortisone was discontinued.

On January 9, 1953, the patient was readmitted to the hospital acutely and severely ill. The mother stated that he had been perfectly well until a week prior to this admission, at which time he suddenly became ill with chills and fever. Delirium, prostration, and jaundice developed rapidly. He was given antibiotics and phenobarbital. The symptoms increased and the patient became semicomatose. Physical examination revealed a well nourished but markedly jaundiced child. The liver border was palpated 4 inches below the costal margin. The abdomen was distended.

Laboratory findings showed a hemoglobin level of 11.5 gm. per cent; erythrocytes, 4,470,000 per cmm.; white blood cells, 16,450 per cmm. with the differential showing 48 neutrophils, 2 eosinophils, 44 lymphocytes, and 6 monocytes. A cephalin-cholesterol flocculation test was 4 plus at 24 hours. The indirect bilirubin was 20.6 mg. per cent. A chest roentgenogram was normal.

Respirations were rapid. He remained semicomatose. Occasionally, bright red blood ran from his nose and mouth. He was placed in oxygen, given whole blood, infusions of glucose, water-soluble vitamins, vitamin K, gamma globulin, penicillin, and large doses of cortisone. Within 12 hours the temperature (rectal) rose from 97° F. to 101.2° F. Fifteen hours after admission to the hospital, and approximately 7 days after the onset of fever and jaundice, the patient expired in hepatic coma. Clinically, it was thought that death occurred as a result of an overwhelming homologous serum hepatitis contracted from one of the several blood transfusions he had received.

NECROPSY FINDINGS

Necropsy was performed 3 hours after death. The skin and sclerae showed severe jaundice. The mouth contained clotted blood. The abdomen was distended.

Peritoneal Cavity. The panniculus measured approximately 1.0 cm. in thickness. The peritoneal cavity contained a small quantity of yellow serous fluid. Occasional subserosal petechiae were seen on the external surfaces of the small intestine. The liver extended 8 cm. below the costal margin in the right midclavicular line. The spleen extended 4 cm. below the costal margin.

Thoracic Cavity. There were occasional pleural petechiae. The

pericardial cavity contained approximately 5 cc. of yellow serous fluid. The trachea and large bronchi contained a small quantity of blood-stained mucus. No source of bleeding was demonstrated in the oropharynx.

Heart. The heart weighed 60 gm. (normal, 56 gm.). No anomalies were present.

Lungs. The right lung weighed 130 gm. (normal, 80 gm.) The left lung weighed 105 gm. (normal, 75 gm.). Both lungs were crepitant. The cut surface was mottled pink. A small amount of frothy blood-stained material was present in the lumen of the small bronchi. Occasional focal hemorrhagic areas, 4 to 5 mm. in diameter, were scattered throughout the lung parenchyma.

Spleen. The spleen weighed 130 gm. (normal, 30 gm.). It was firm with rounded edges, and the cut surface was dark reddish purple. The malpighian corpuscles were prominent. The trabeculae were indistinct. The red pulp scraped away with ease.

Liver. The liver was soft. The external surface was a mottled tan and yellow-brown. Tiny yellow-white nodules which measured up to 3 mm. in diameter were seen widely scattered beneath the capsule. On close inspection they were noted to have irregular borders. The cut surface of the liver was yellow mottled with dark red areas. The above-mentioned nodules were scattered in a random fashion throughout the parenchyma.

Gallbladder. The gallbladder contained approximately 5 cc. of yellow-green bile.

Pancreas. The pancreas was light tan and normal in size, shape, and consistency.

Adrenal Glands. The combined weight of the adrenal glands was 8 gm. The cut surface was normal.

Genito-urinary System. The kidneys were symmetric, each weighing 70 gm. (normal, 44 gm.). The capsules stripped with ease revealing smooth surfaces that were yellowish pink. The remainder of the genito-urinary tract was normal.

Gastro-intestinal Tract. The stomach contained a small quantity of coffee-ground material mixed with mucus. Similar material was present in the intestine. No parasites or ova were found in samples of the feces taken from numerous sites of the gastro-intestinal tract. The mucosa was pale throughout, except for minute areas of ecchymosis in the stomach and duodenum. The mesenteric lymph nodes were soft, brownish red, and measured up to 1.5 cm. in diameter.

Brain. The fresh brain weighed 1,100 gm. (normal, 1,050 gm.). The

dura was smooth and glistening, and the major sinuses were free of thrombi. The pituitary gland was normal and the middle ears contained no exudate. The leptomeninges were smooth and transparent and the leptomeningeal vessels were congested. There was narrowing of the sulci and flattening of the gyri. Multiple coronal sections through both cerebral hemispheres revealed good differentiation between the gray and the white matter. The gray matter had the translucent appearance characteristic of cerebral edema. The ventricular system was normal. No lesions were noted in the parenchyma of either hemisphere. The aqueduct of Sylvius was patent. Transverse sections of the brain stem and cerebellum revealed no abnormalities except an area of hemorrhage approximately 3 mm. in diameter adjacent to the dentate nucleus in the right cerebellar lobe.

Microscopic Findings

All tissues were preserved in 10 per cent formalin. Sections were stained routinely with hematoxylin and eosin, Giemsa's stain, and by the periodic acid-Schiff procedure (PAS). Representative lesions were sectioned serially.

Heart. In the heart a few scattered areas of hemorrhage were present in the epicardial fat. In one section from the myocardium of the left ventricle there was a circumscribed granulomatous lesion infiltrated with lymphocytes, plasma cells, and occasional eosinophils. A few Anitschkow cells were in the periphery. Within the center of the lesion there were several multinucleated giant cells of the foreign body type. Serial sections through this lesion revealed a longitudinal fragment of a larval parasite. Other sections showed occasional focal areas of interstitial myocarditis. The infiltrate in these areas was composed of lymphocytes and a few large macrophages.

Lung. In scattered areas of the lung the alveolar septa were thickened by congestion, edema, and cellular infiltrate of large mononuclear cells. Within some of the thickened septa there were a few large multinucleated macrophages. The bronchi contained a small quantity of mucus. Edema fluid was present in the alveolar spaces. Scattered throughout the lung parenchyma were focal granulomas (Figs. 1 and 2). These were composed of a central area of fibrinoid necrosis rimmed by epithelioid cells, eosinophils, and lymphocytes. Many granulomas contained fragments of nematode larvae cut in various planes. When the larvae were coiled upon themselves, more than one larval segment was seen. The larvae were PAS positive and some PAS positive débris

was present in the necrotic centers of the granulomas. Considerable fibrosis was present about the periphery of each lesion.

In one section there was a granuloma adjacent to a bronchus, the overlying epithelium of which was ulcerated. One field showed immediately beneath the pleura a granuloma which contained a fragment of a larval parasite (Figs. 3 and 4). Granulomas in which there were numerous foreign body giant cells usually contained no larvae. Rarely were the granulomas in apposition. No larvae were identified within the multinucleated giant cells. An occasional lesion was obliterated by fibrosis.

Liver. Sections of liver removed at necropsy (Figs. 6 and 7) showed complete necrosis of the hepatic cells in most areas and a diffuse cellular infiltrate of lymphocytes, neutrophils, and plasma cells. Bile thrombi were present in the bile ducts. A granulomatous lesion within the capsule was composed principally of epithelioid cells and lymphocytes and contained a parasite. The collagen fibers were disrupted and fragmented. Numerous granulomas were present throughout the liver (Figs. 8, 9, 10, and 11). A granuloma containing a larva involved the wall of a large intrahepatic branch of the portal vein (Figs. 12 and 13). Occasionally, the entire parasite was surrounded by epithelioid cells. Well preserved larvae were surrounded by granular fibrinoid material. Degenerating larvae were surrounded by foreign body giant cells, epithelioid cells, lymphocytes, and eosinophils. The presence of extensive parenchymal necrosis failed to change fundamentally the histologic character of the granulomas. The parasites and their fragments stained dark red in the PAS preparations and PAS-positive débris was present in the fibrinoid material of the granulomas (Figs. 14 and 15).

Spleen. The splenic sinusoids were congested and contained eosinophils and neutrophils. The germinal centers were prominent, and occasionally showed a toxic reaction center.

Pancreas. There were focal areas of lymphocytic infiltration within the pancreatic stroma. One section revealed a granuloma similar to those described previously. Serial sections failed to disclose a larva.

Kidney. Immediately beneath the renal capsule and lying within the cortex was a granuloma similar to those in the liver and lungs. It was approximately twice the diameter of a glomerulus and a tiny distorted fragment of the parasite was within its center.

Large Bowel. Immediately beneath the mucosa of the large bowel

there was a granuloma which contained a larva (Figs. 16 and 17). Small nuclear fragments and cellular débris were present about the parasite. Lymphocytes and plasma cells were found in the fibrous tissue of the periphery. Eosinophils were scanty.

Small Bowel. A granuloma within the muscularis of the small bowel contained a well preserved parasite.

Mesenteric Lymph Nodes. There was a moderate amount of reticuloendothelial hyperplasia in the mesenteric lymph nodes and the germinal centers were prominent. Two of the nodes sectioned (Figs. 18, 19, and 20) contained granulomas with larval parasites.

Common Bile Duct. A lymph node adjacent to the common bile duct contained a granuloma. Serial sections failed to reveal the parasite.

Bone Marrow. The bone marrow from the body of the vertebrae was hyperplastic. There was a predominance of eosinophils in all states of maturation. Other cellular elements were normal.

Sections from the diaphragm, urinary bladder, testes, adrenal glands, thyroid gland, esophagus, stomach, gallbladder, cecum, and appendix revealed no granulomas and were normal histologically.

Brain. Sections of brain and spinal cord showed granulomas with and without larvae in the following locations: Spinal cord (Figs. 21, 22, and 23), pons, cerebellar peduncle (Figs. 24, 25, 26, 27, 28, and 29), cerebral cortex (Figs. 30, 31, 32, 33, 34, and 35), and within the interstices of the leptomeninges (Figs. 33 and 34). These granulomas were similar to those described previously. They contained numerous giant cells, eosinophils, plasma cells, and lymphocytes (Fig. 35). In addition, there was a proliferation of epithelioid cells and microglial cells. Blood vessels in the general vicinity of the lesion showed perivascular eosinophils and macrophages. Adjacent nerve cells were normal. The leptomeninges overlying the granulomas showed an infiltrate of macrophages and eosinophils.

Comments

The pertinent features in this case are similar to those of previous reports in which eosinophilia, constitutional symptoms, and hepatomegaly were the outstanding clinical manifestations of a prolonged, well defined syndrome.[6,11,12] Widespread dissemination of the larvae was present with an accompanying focal granulomatous reaction. None of the lesions were visible grossly, with the exception of those in the liver. Therefore, the degree of infestation is indicated by the many granulomatous foci found fortuitously in routine sections, as well as by the quantitative recovery of larvae as reported in the next section.

Recovery of Larvae

Using press preparations of fresh tissue removed from this patient at necropsy, quantitative estimations of the number of larvae present in the skeletal and cardiac muscle, liver, and brain were made. Approximately 15 gm. of fresh liver and 8 gm. of fresh skeletal muscle (from various locations) were digested in pepsin using a technique as follows: Freshly prepared 1 per cent pepsin solution, adjusted to pH 2 with hydrochloric acid, was added along with selected tissue specimens to a Waring blendor cup. Twenty-five cc. of pepsin solution was employed for each 2 gm. of tissue. After thorough comminution, the mixture, transferred to conical sedimentation flasks, was incubated overnight (about 15 hours) at 37° C. With satisfactory digestion, the larvae collected in the bottom of the sedimentation flasks permitting decantation of the supernatant fluid. The remaining digest in each flask was washed with tap water several times and concentrated further by centrifugation before microscopic examination.

From the liver, 60 larvae per gm. were recovered; from the muscles, 5 larvae per gm. All of the larvae were motile and identifiable as *T. canis*. The recovery of the larvae of Toxocara from the pepsin digests of striated muscles and liver suggests that this method might be applied to biopsy specimens as a diagnostic procedure in suspected cases. Using a hand lens, minute lesions were seen in the skeletal and cardiac muscle from which motile larvae were recovered by the use of press preparations. In and around all encapsulated lesions there were Charcot-Leyden crystals. Press preparations from various areas of the brain revealed motile larvae. It was estimated that there were from 3 to 5 larvae per gm. of brain tissue.

Discussion

When seen in press preparations or in pepsin digests, infective second stage Toxocara larvae exhibit a high degree of activity characterized by rapid flexion in the dorsoventral plane. They have little or no progressive motility in a medium containing little solid material. *T. canis* larvae removed from experimentally infected abnormal hosts and from the case presented in this paper ranged in length from 360 to 440 μ, and in maximum diameter from 18 to 22 μ. Under low magnification (Fig. 36), each larva can be divided roughly into two portions: a clear esophageal region occupying less than one third of the total length and a more dense intestinal region packed with refractile globules and terminating about 60 μ anterior to the tip of the tail. The larvae are elongated cylindric organisms with the body having almost parallel lateral margins as seen in the optical plane. The anterior quar-

ter of the body tapers equally to a three-lipped, subterminal, dorsally inclined mouth. Anterior to the mouth a sharp spine-like cuticular thickening is found on the ventral margin of the buccal capsule. This latter oral structure is quite characteristic of the early second-stage Toxocara larvae and facilitates their identification in pepsin digests and in press preparations (Fig. 36). The posterior end is tapered more abruptly, commencing anterior to the termination of the intestine and continuing to a slender attenuated tail.

In stained sections, *T. canis* larvae can be identified accurately by the use of several morphologic characters not shared with other nematodes likely to be encountered in human tissues. In transverse sections through most levels of the larva single lateral alae are formed by sharp foldings of the cuticle in the lateral line (Figs. 22, 23, 25, and 35). The alae are more pronounced at the level of the posterior esophagus and at all intestinal levels.

Two columnar, eosinophilic, non-nucleated structures, the excretory columns, extend in the body cavity of the intestinal and posterior esophageal regions. These latter structures commonly fill the body cavity, compressing the intestinal cells dorsad (Figs. 22, 23, and 35). The intestine is without lumen and consists of seven elongated cells arranged in linear fashion (Figs. 13 and 34). The intestine frequently is difficult to recognize except in transverse section (Fig. 23). However, in longitudinal section minute granules associated with the cytoplasm of the intestinal cells are commonly observed. These granules stain with both Giemsa's stain (Fig. 7) and hematoxylin (Fig. 23). Longitudinal and transverse sections of *T. canis* at esophageal levels furnish fewer diagnostic characters and may be confused with similar sections of larvae of *A. lumbricoides* and of hookworms. All of these have in common a slender esophagus with an enlarged terminal portion surrounded throughout most of its length by ganglionic nuclei (Figs. 7, 9, 11, and 20). The nerve ring appears similar in these forms and consists of eosinophilic fibers which displace the ganglionic nuclei and encircle the esophagus near its mid-portion (Figs. 9 and 20). Median sagittal sections of the buccal apparatus demonstrate the characteristic ventral cuticular spine-like structure of *T. canis* and are diagnostic (Figs. 17 and 20).

Therefore, single, well stained, transverse sections through most levels of the intestine are sufficient for specific diagnosis of Toxocara. Larvae with diameter greater than 22 μ, but having similar internal organization, may be those of *A. lumbricoides* or hookworm, since *T. canis* larvae do not appear to undergo growth or differentiation in the human host. If adequate transverse sections of the intestinal

region are not available, reconstruction of the parasite may be necessary. Diagnosis in this case makes use of the minute nuclear organization and organ relationships of the larvae. The detailed anatomy of Toxocara, *A. lumbricoides,* hookworm, and other tissue-invading larvae will be made available in two forthcoming publications.

The histologic structure of the granulomas was similar in all organs, although the larvae were not identified in each lesion. The larvae, when present, were surrounded by the tissue resulting from a focal granulomatous reaction, infiltrated with neutrophils and eosinophils. Fibrinoid necrosis occurred in the central zone of recent lesions. Older lesions showed fibrous encapsulation. Foreign body giant cells, epithelioid cells, macrophages, and lymphocytes were present about degenerating larvae. No necrotizing angiitis was observed.

Granulomas were most frequent in the liver and in the lungs; however, similar lesions were observed in cardiac muscle, liver capsule, muscularis of the small bowel, beneath the serosal surface of the large bowel, renal cortex, pancreas, mesenteric lymph nodes, spinal cord, pons, cerebellar peduncle, and cerebral cortex.

Based on previous clinical observations, it is not likely that this case is unique in the number of larvae in the viscera, or in the anatomical distribution of the granulomas. Presumably, this patient was not re-exposed to the larvae after his discharge from the hospital 6 weeks prior to death. A general state of well being was experienced from the date of the hospital discharge until the onset of the fatal fulminating hepatitis.

Because of the minute size and widespread distribution of the granulomas, x-ray examinations during the terminal illness failed to demonstrate their presence, although numerous lesions containing larvae were found in the lungs on histologic examination.

Granulomas containing the larvae in the central nervous system may explain the neurologic manifestations which occasionally occur during the course of visceral larva migrans. Similar neurologic symptoms have been reported in children with intestinal ascariasis but the relationship remains obscure.[13,14] Beautyman and Woolf,[15] in 1951, reported an "ascaris larva" surrounded by granulomatous inflammation in the thalamus of a child who died from acute poliomyelitis. The granuloma they described is identical histologically with the granulomas found by us in the brain. The reconstructed larva was 330 to 335 μ long and had a maximum diameter of 15 to 20 μ. The dimensions and structure are consistent with a *T. canis* larva.

No satisfactory explanation has been proposed for the protean

neurologic symptoms that occur in human ascariasis, but mixed infections with Toxocara and *A. lumbricoides* may have been overlooked. The demonstration of the invasion of the central nervous system by the larvae of Toxocara indicates that these larval parasites, and not the adult ascarids within the intestinal lumen, may be the agents responsible for neurologic manifestations. It is probable that the larvae of Toxocara invade all tissues of the human host more readily than do the larvae of *A. lumbricoides*. Some of the nematodes found in the human eye by Wilder[16] can be identified as the larvae of Toxocara. A study of the available material from Wilder shows the structure of the larvae to be identical with that of Toxocara and the granulomas to be identical with those described in the present report.

The cosmopolitan distribution of Toxocara, and the likelihood of its presence being overshadowed by more conventional parasites, indicate that infection with this larval form may occur more frequently than previously supposed. The close association of young children with dogs and cats establishes an ideal set of circumstances under which infective ova may be ingested readily.[17] Clinical and pathologic observations have led us to the conclusions that the degree of eosinophilia and the severity of the clinical symptoms are related roughly to the number of ova ingested.[18] In addition, we have observed eosinophilia in the siblings of patients with severe infections in which the larvae of *T. canis* were identified in liver taken for biopsy. If the patients had not had infections sufficiently severe to warrant liver biopsy in search for the cause of their illness, a diagnosis of "familial eosinophilia" doubtless would have been made.

The constitutional symptoms, the visceral granulomas, and the eosinophilia in the blood and bone marrow characterize a definite clinical syndrome. Species identification of the invading nematode is not necessary in order to establish a presumptive clinical diagnosis. As yet, no specific antigen for agglutination or diagnostic skin test is available.[19] The minute size of the lesions and their spontaneous healing by fibrosis account for the usual favorable prognosis, even in heavy infections.

Summary

A 19-months-old white male with visceral larva migrans died of an incidental homologous serum hepatitis. At necropsy, granulomas, with and without the larvae of *Toxocara canis*, were widely scattered throughout the viscera and central nervous system.

Quantitative estimates of the number of motile larvae present in

the liver, brain, and skeletal muscle were made by using aliquot samples of fresh tissue removed at necropsy. Press preparations and pepsin digestion studies revealed the liver to contain approximately twelve times as many larvae per gram as the skeletal muscle, and approximately twenty times as many larvae per gram as the brain.

The presence of larvae within the central nervous system suggested that they might be responsible for some of the puzzling neurologic disturbances often encountered clinically in patients with visceral larva migrans. A review of available material previously reported by Wilder[16] and by Beautyman and Woolf[15] suggests that the larvae found by them in the human eye and within the human brain, respectively, were the larvae of Toxocara.

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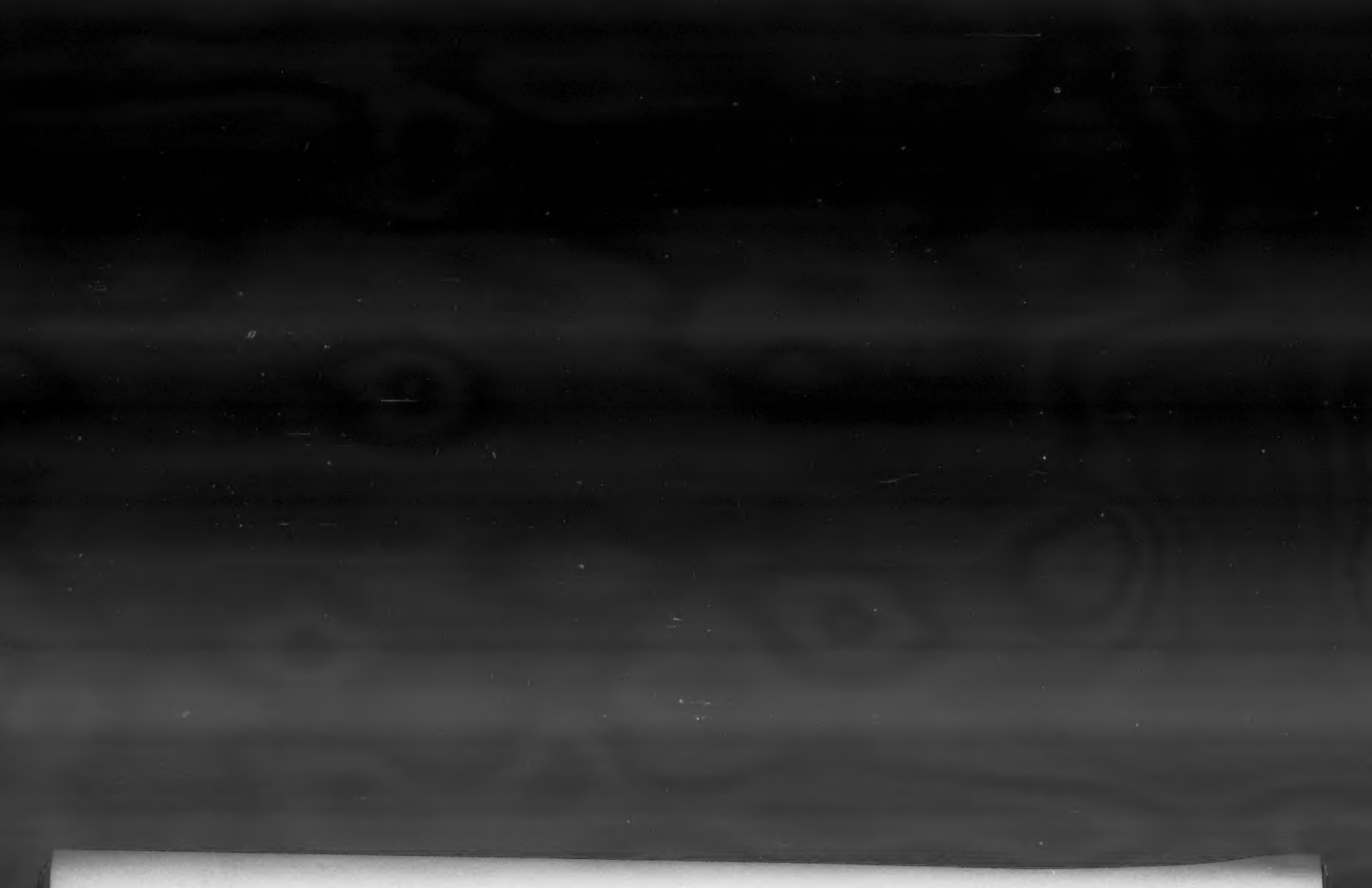
LEGENDS FOR FIGURES

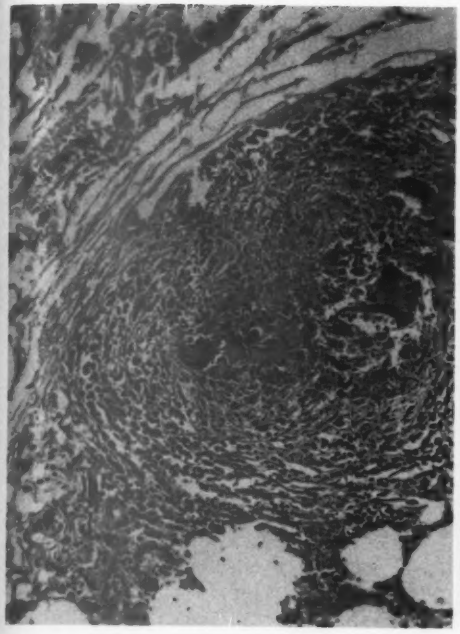
FIG. 1. Lung. Granuloma showing a transverse fragment of a Toxocara larva. A moderate degree of fibrosis is present. The granuloma measured 640 by 520 μ. Hematoxylin and eosin stain. × 126.

FIG. 2. Higher magnification of Figure 1. The larva has been sectioned transversely at a level immediately posterior to the termination of the intestine. The lateral alae are not evident. The diameter of the larva at this level was 14 μ. × 565.

FIG. 3. Lung. Subpleural granuloma containing a longitudinal fragment of a Toxocara larva. The granuloma measured 820 by 520 μ. Hematoxylin and eosin stain. × 126.

FIG. 4. Higher magnification of Figure 3. The larva is sectioned at a level of the intestine. Only the excretory columns and ventral-line nuclei are evident. × 565.





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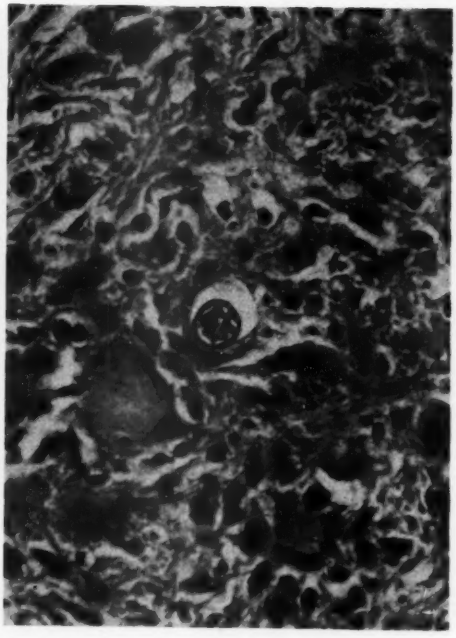

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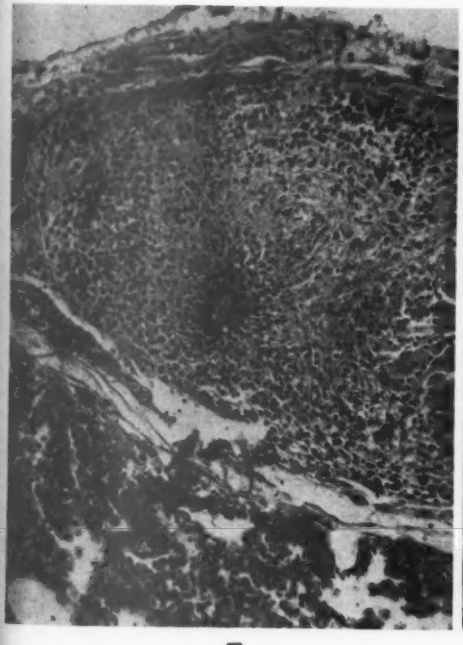

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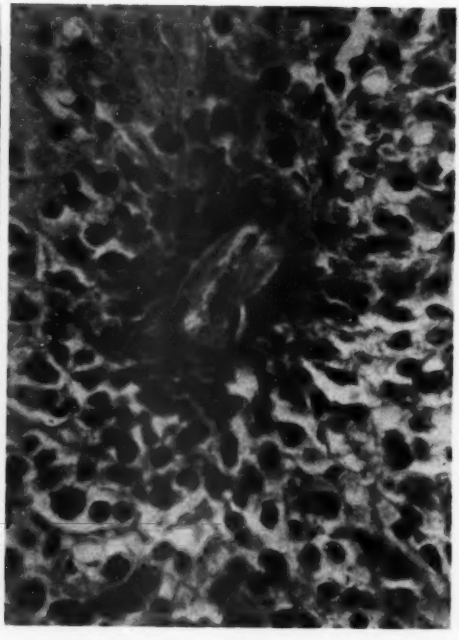

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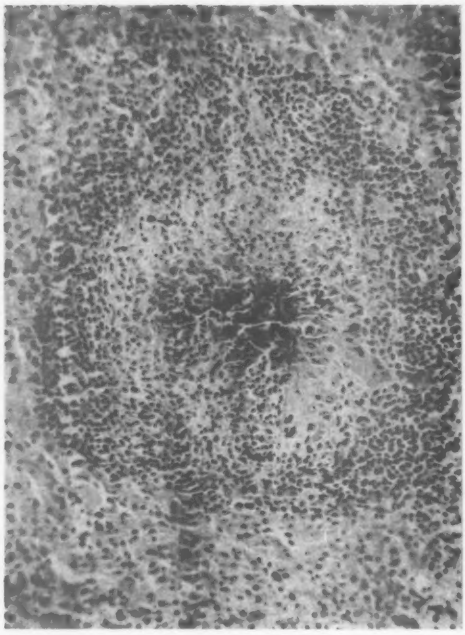

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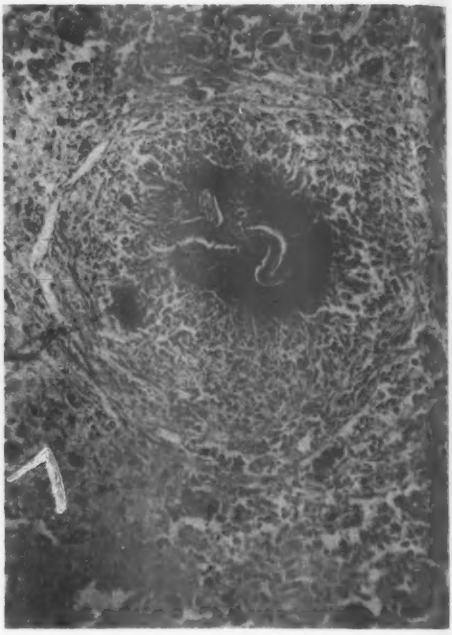

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FIG. 5. Liver, biopsy specimen. Granuloma showing a central area of necrosis, epithelioid cells, foreign body giant cells, and a dense infiltration of eosinophils and neutrophils about the periphery. Normal liver cells may be noted. A transverse section of a Toxocara larva is present near the center of the lesion. Hematoxylin and eosin stain. × 126.

FIG. 6. Liver, necropsy specimen. Longitudinal fragment of a Toxocara larva is present in a granuloma which is surrounded by fibrous tissue. There is marked necrosis of liver cells, as a consequence of incidental homologous serum hepatitis. The granuloma measured 470 by 470 μ. Giemsa's stain. × 126.

FIG. 7. Higher magnification of Figure 6. Median-sagittal section of the larva showing the esophagus, the first intestinal cell, and the excretory cell nucleus. × 565.

FIG. 8. Liver, necropsy specimen. Granuloma containing fibrinoid necrosis, epithelioid cells, lymphocytes, and a few eosinophils. A longitudinal section of a Toxocara larva is present. The granuloma measured 520 by 430 μ. Hematoxylin and eosin stain. × 126.

FIG. 9. Higher magnification of Figure 8. Longitudinal section of the larva showing dense ganglionic nuclei, nerve ring, posterior portion of the esophagus, and an excretory column. Width at esophageal termination was 18 μ. × 565.

FIG. 10. Liver. Granuloma containing oblique section of a single Toxocara larva at the junction of the esophagus and intestine. The granuloma shows foreign body giant cells with an infiltration of lymphocytes and plasma cells about the periphery. The granuloma measured 520 by 470 μ. Hematoxylin and eosin stain. × 126.

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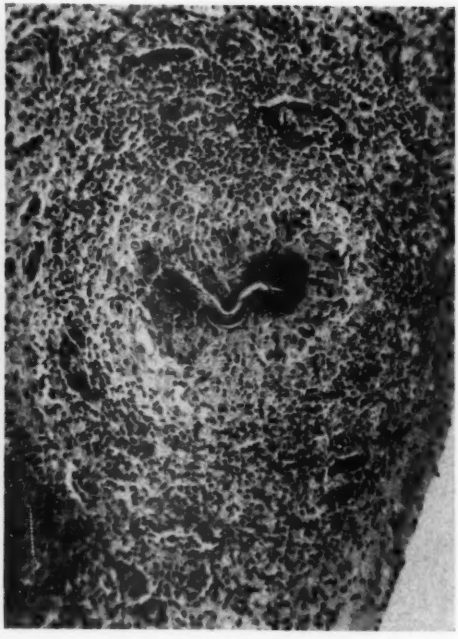

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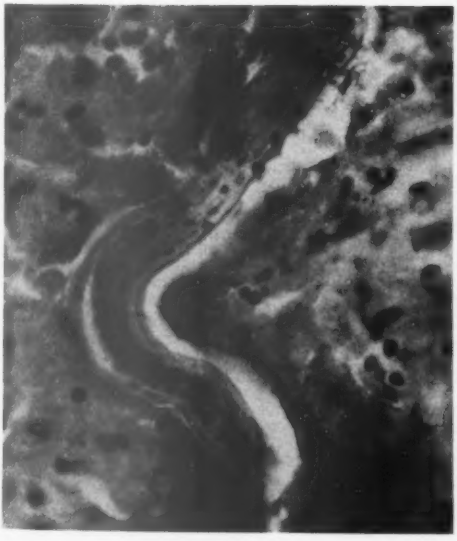

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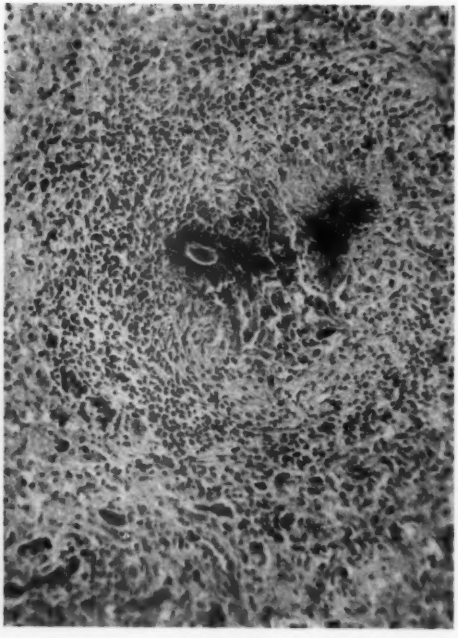

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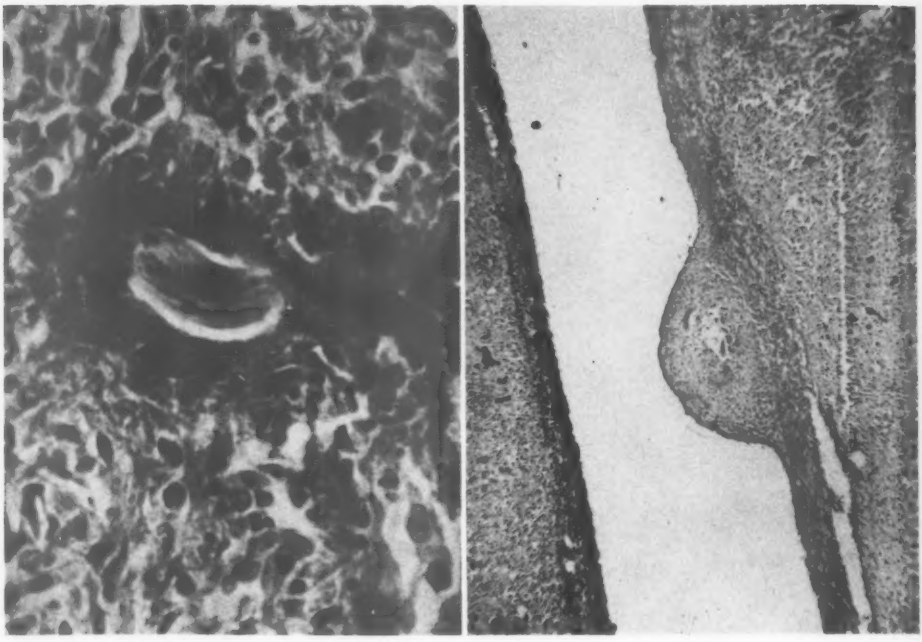

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FIG. 11. Higher magnification of Figure 10. Oblique section through the larva at the level of posterior esophagus. The diameter of the larva at this level was 18 μ. × 565.

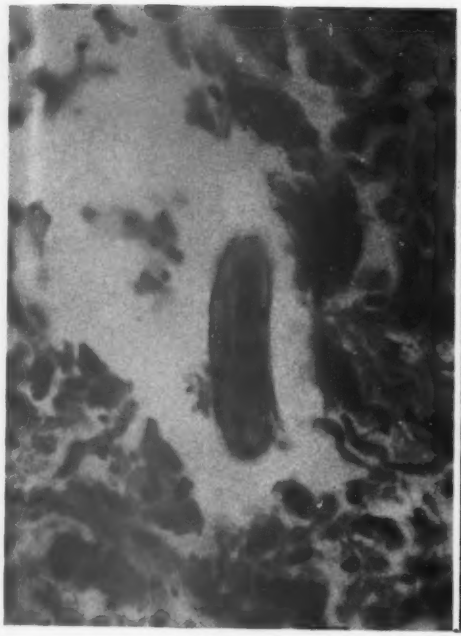
FIG. 12. Intrahepatic branch of the portal vein. Granuloma attached to the lateral wall containing a longitudinal fragment of a Toxocara larva. The granuloma measured 600 by 390 μ. Hematoxylin and eosin stain. × 49.

FIG. 13. Higher magnification of Figure 12. Longitudinal section of the Toxocara larva through a level of the intestine. Diameter at this level was 16 μ. Only the large paired excretory columns are evident. × 565.

FIG. 14. Liver. Granuloma showing longitudinal fragment of a Toxocara larva. Note Schiff-positive débris and larva in the necrotic center. The granuloma measured 560 by 560 μ. Periodic acid-Schiff's (PAS) stain. × 126.

FIG. 15. Higher magnification of Figure 14. × 565.

FIG. 16. Large bowel. Submucosa with a granuloma containing a longitudinal fragment of a Toxocara larva. The granuloma is composed principally of epithelioid cells with few eosinophils in the periphery. Hematoxylin and eosin stain. × 126.



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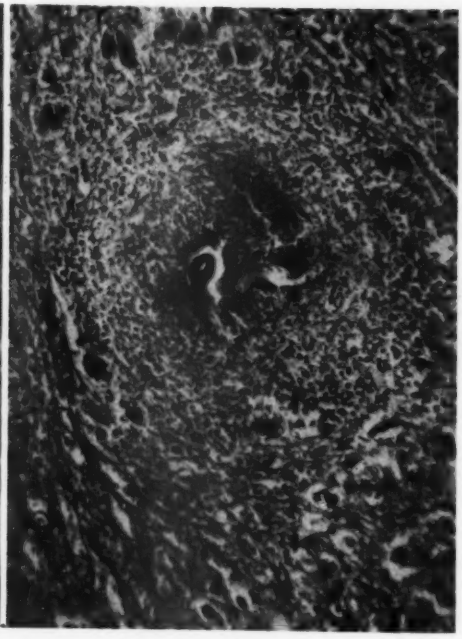

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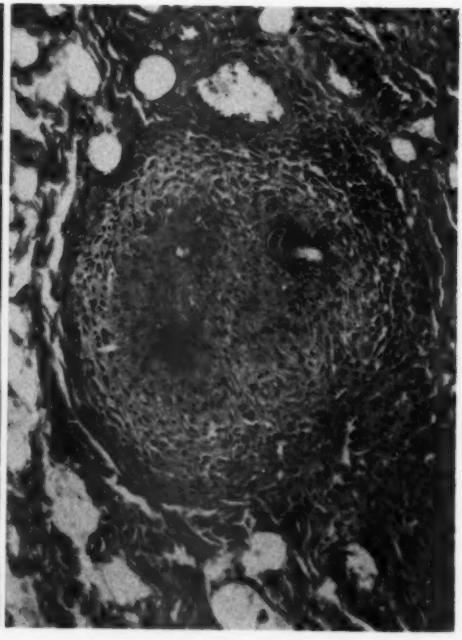

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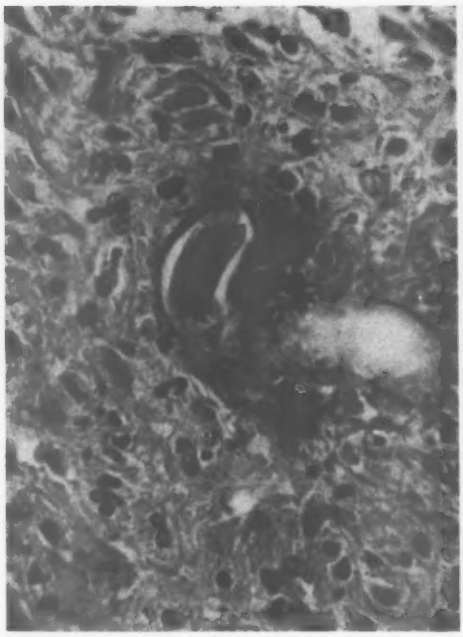

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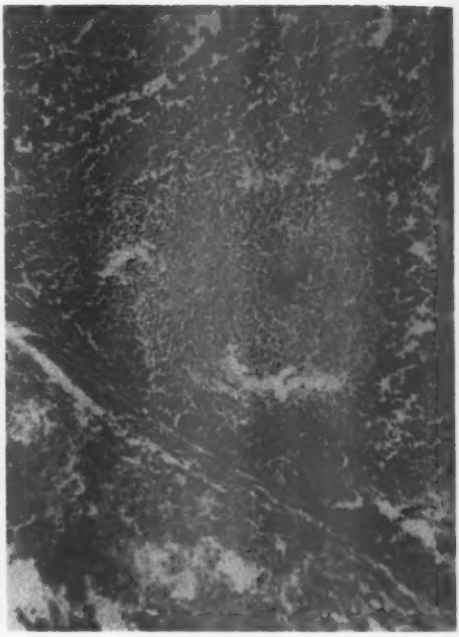

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FIG. 17. Higher magnification of Figure 16. Median sagittal section through anterior tip of the larva showing the buccal capsule and the characteristic ventral cuticular thickening. × 565.

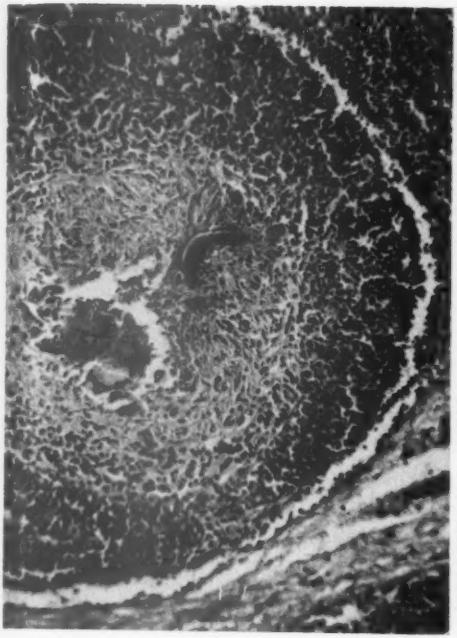
FIG. 18. Mesenteric lymph node. A focal area of fibrosis is present but no larva is seen. Hematoxylin and eosin stain. × 126.

FIG. 19. The fifth section obtained on serial section of the lesion in Figure 18. A granuloma is present containing foreign body giant cells and a Toxocara larva. Hematoxylin and eosin stain. × 126.

FIG. 20. Higher magnification of Figure 19. Longitudinal section of the anterior portion of a larva showing the characteristic buccal structure, esophagus, nerve ring, and ganglionic nuclei. × 565.

FIG. 21. Upper cervical spinal cord. Posterior column. Granuloma showing a transverse section of a Toxocara larva and perivascular infiltration. The granuloma measured 430 by 520 μ. Hematoxylin and eosin stain. × 126.

FIG. 22. Higher magnification of Figure 21. Transverse section of the larva through the intestinal region, showing lateral alae, paired excretory columns, and a central intestinal cell compressed between the excretory columns. The diameter at this level was 18 μ. × 565.



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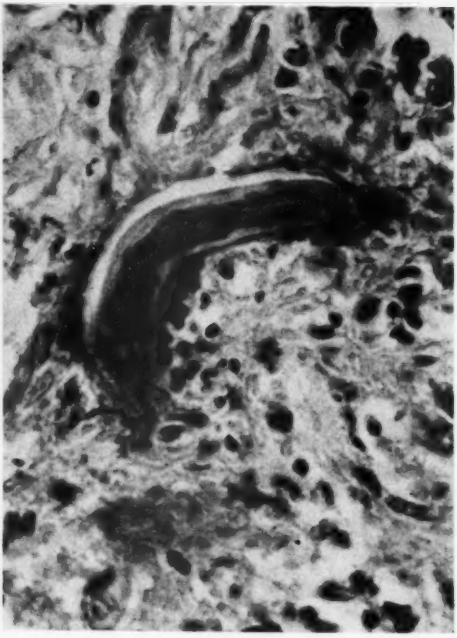

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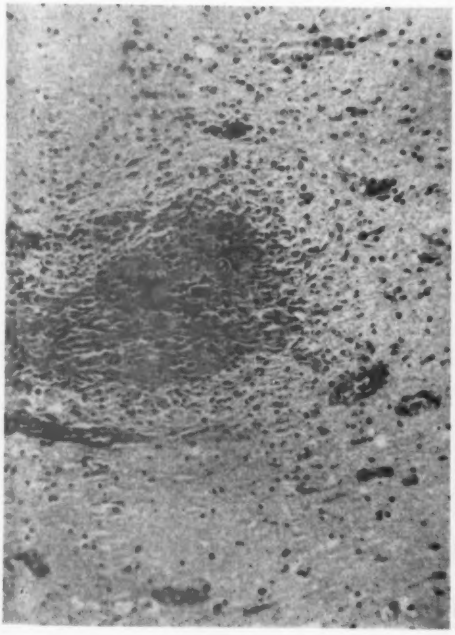

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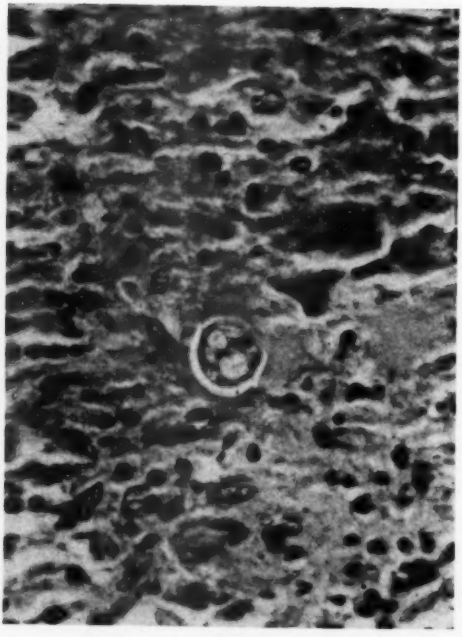

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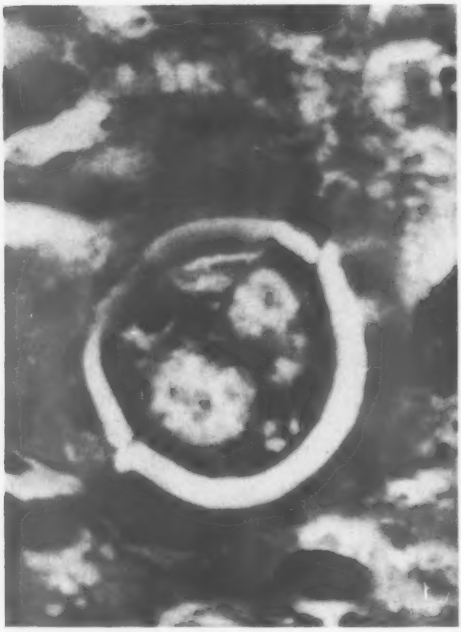

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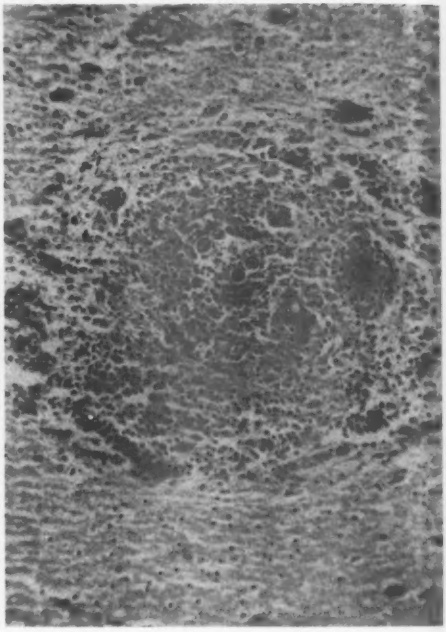

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FIG. 23. Oil immersion of Figure 21. The intestine contains one cell and has no lumen. The lateral alae are prominent. The body of the cell is outlined by small basophilic granules. × 2,070.

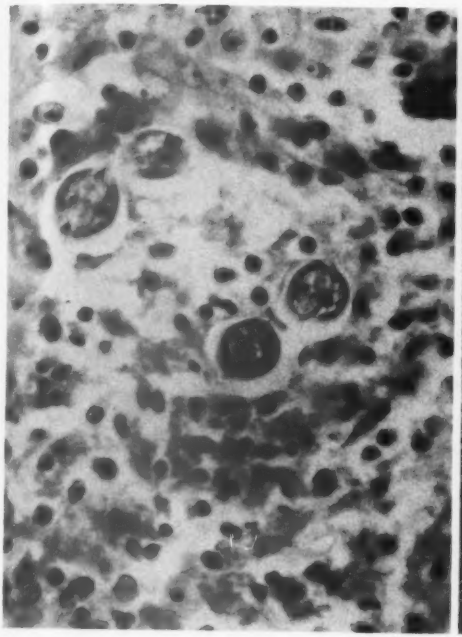
FIG. 24. Cerebellar peduncle. Granuloma containing four transverse fragments of a single Toxocara larva. Foreign body giant cells, macrophages, lymphocytes, and eosinophils about the periphery and in perivascular spaces. The granuloma measured 430 by 430 μ. Hematoxylin and eosin stain. × 126.

FIG. 25. Higher magnification of Figure 24. Three sections of the larva are at the level of the intestinal region and show lateral alae and paired excretory columns. One section is through the esophageal region showing the central esophagus surrounded by ganglionic nuclei. Maximum diameter was 20 μ. × 565.

FIG. 26. Cerebellar peduncle. Granuloma containing two fragments of a Toxocara larva. Perivascular infiltrate containing eosinophils in the immediate vicinity. The granuloma measured 520 by 430 μ. Hematoxylin and eosin stain. × 126.

FIG. 27. Higher magnification of Figure 26. Tangential section of the larva at the esophageal region and a longitudinal section of intestinal region showing the excretory columns and characteristic basophilic granules of the intestinal cells. × 565.

FIG. 28. Cerebellar peduncle. Granuloma containing a Toxocara larva in the center. There is peripheral fibrosis with some increase in microglia. An infiltrate of lymphocytes, plasma cells, and eosinophils is present. The granuloma measured 600 by 520 μ. Hematoxylin and eosin stain. × 126.



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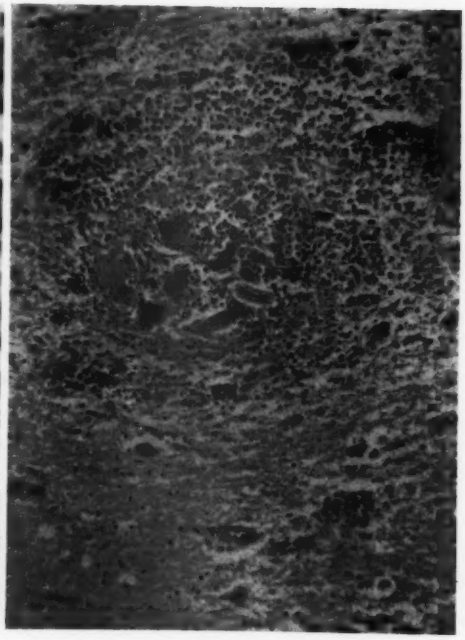

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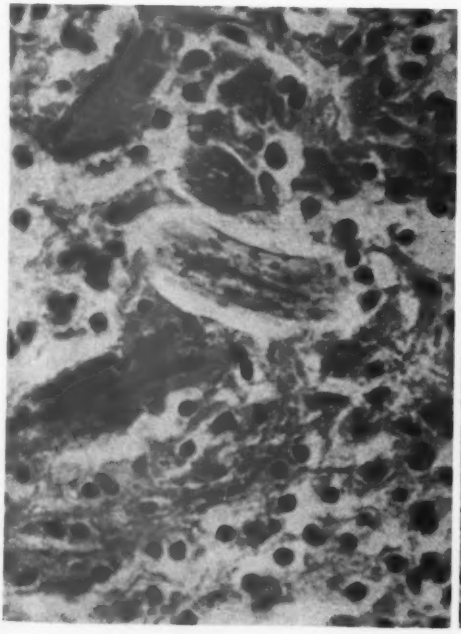

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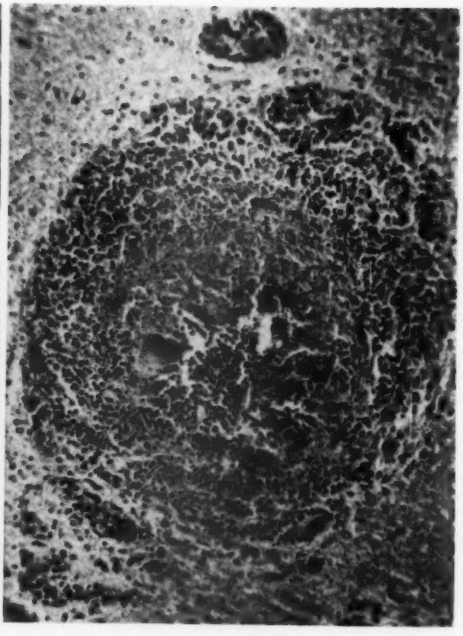

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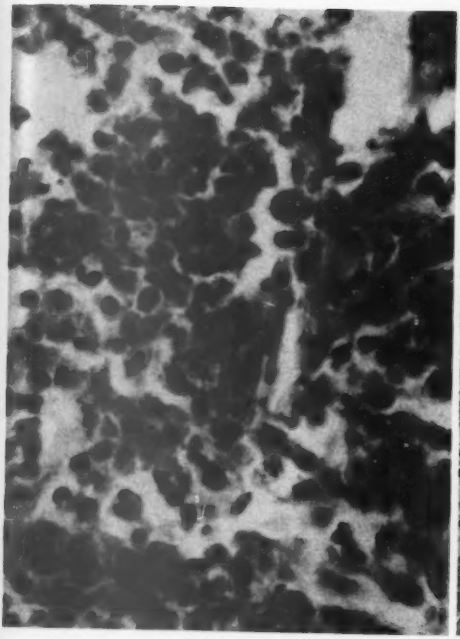
FIG. 29. Higher magnification of Figure 28. × 565.

FIG. 30. Frontal cortex. White matter. Granuloma with foreign body giant cells, epithelioid cells, and an infiltrate of lymphocytes and eosinophils. A distorted fragment of a Toxocara larva cut in longitudinal section is lying near the right margin of the lesion. Hematoxylin and eosin stain. × 126.

FIG. 31. Higher magnification of Figure 30. The larva presents no diagnostic characteristics. × 565.

FIG. 32. Frontal cortex. Granuloma at the junction of the gray and the white matter, containing foreign body giant cells, lymphocytes, and eosinophils. Larval fragments are present near the center of the lesion. Hematoxylin and eosin stain. × 126.



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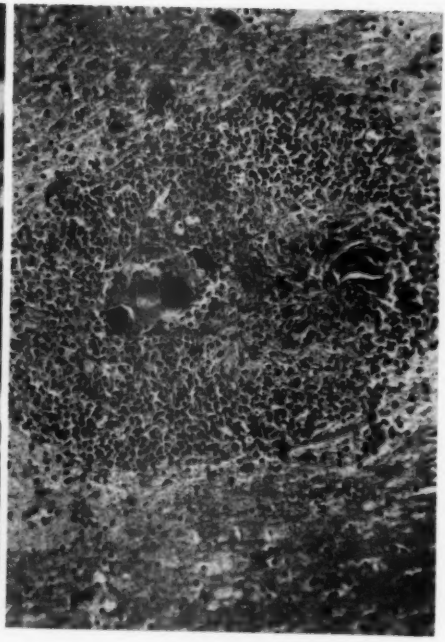

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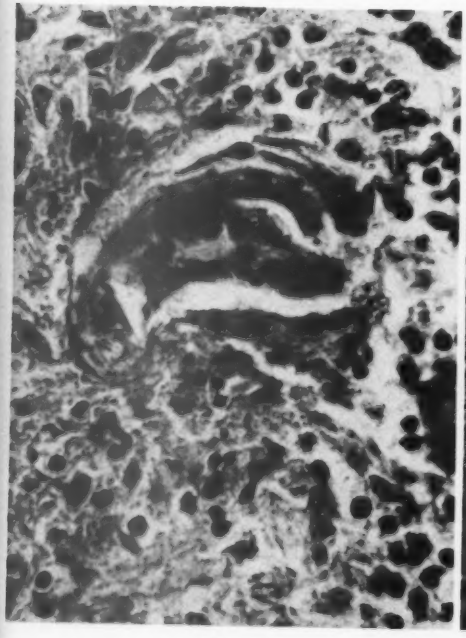

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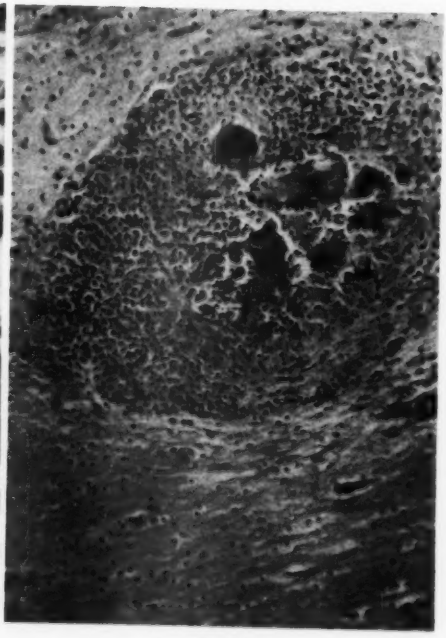

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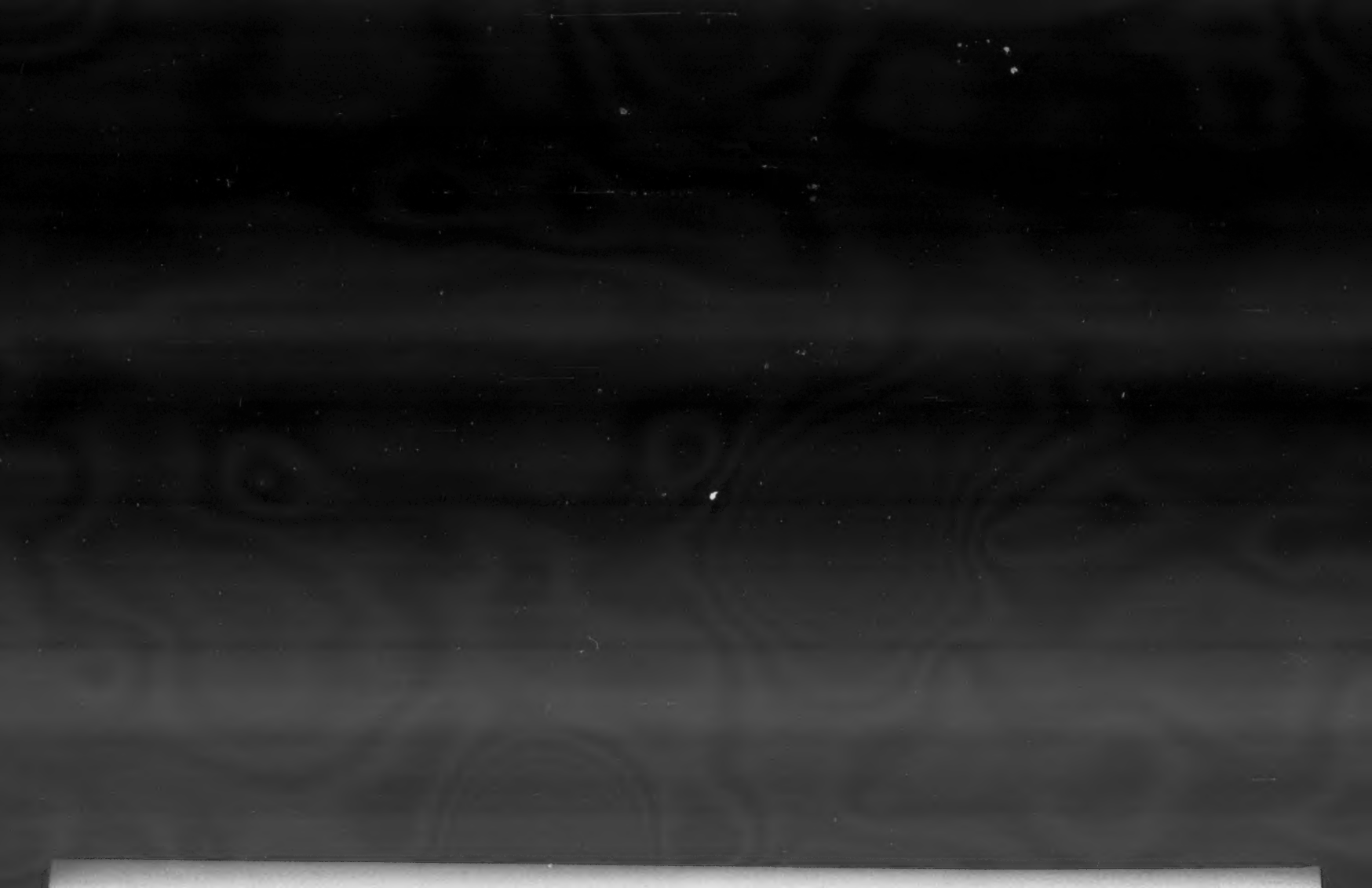
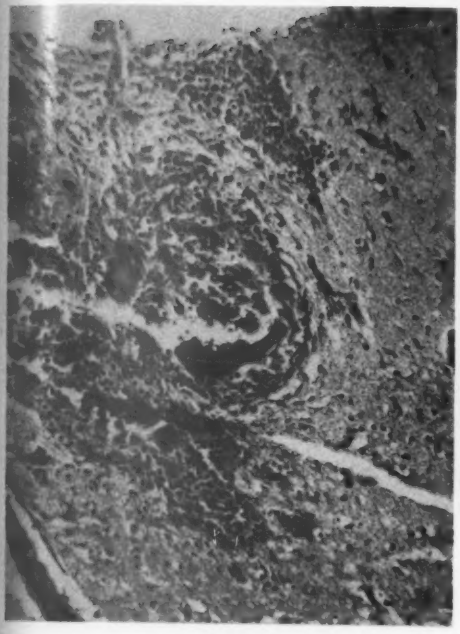
FIG. 33. Cortical gray matter. Superficial layer contains a Toxocara larva cut in longitudinal section. A granuloma contains giant cells, lymphocytes, and eosinophils. The granuloma measured 560 by 520 μ. Hematoxylin and eosin stain. $\times$ 126.

FIG. 34. Higher magnification of Figure 33. Median sagittal section of the larva through the intestinal region, showing the small, regularly spaced, ventral-line nuclei and four large intestinal cell nuclei forced dorsad by the central excretory column mass. Width of the larva was 18 μ. $\times$ 565.

FIG. 35. Frontal cortex. Granuloma in the white matter of the frontal cortex containing a transverse section of a Toxocara larva. There is a focal infiltration of lymphocytes and a focal proliferation of microglia. The larva is sectioned through the intestinal region and shows lateral alae, paired excretory columns, and a central compressed intestinal cell. Diameter of the larva at this level was 20 μ. Hematoxylin and eosin stain. $\times$ 565.

FIG. 36. *Toxocara canis* larva showing the ventral spine-like cuticular thickening of the anterior tip, the clear esophageal region, and refractile intestinal region. Heat-killed, unstained. $\times$ 500.





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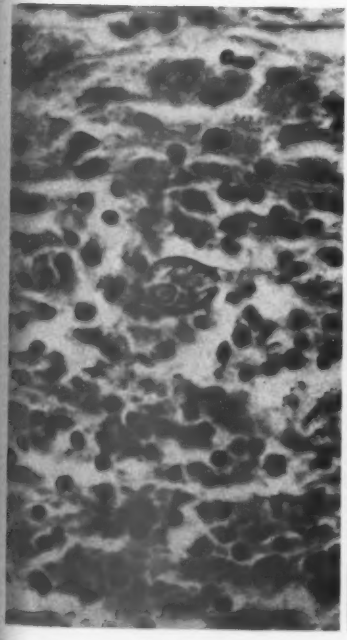

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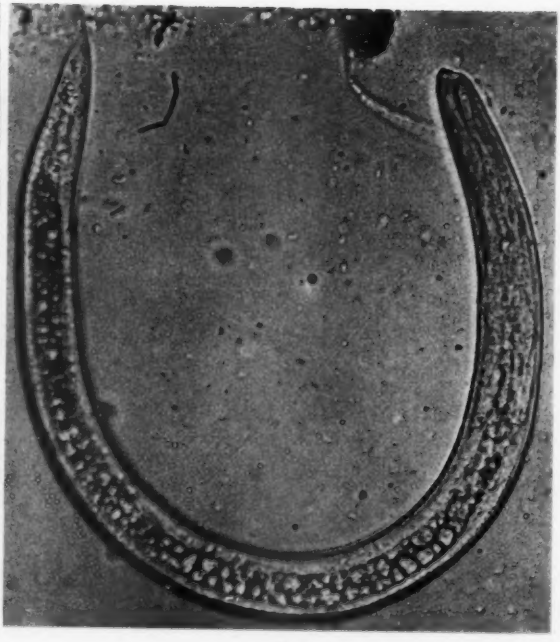

36

THE PATHOLOGY OF ISCHEMIA OF SKELETAL MUSCLE IN MAN

A Description of Early Changes in Muscles of the Extremities Following Damage to Major Peripheral Arteries on the Battlefield*

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During wartime the treatment of vascular trauma is a major concern of the surgeon. Most of the progress in this field has related to the perfection of techniques for optimal restoration of blood flow. Comparatively scant attention has been paid to the striking changes that take place in devascularized tissues. Since the degree of damage to skeletal muscle is of great significance to the surgeon who must decide whether or not to amputate, or what level of amputation to select, a clinicopathologic study of ischemic skeletal muscle was undertaken in soldiers sustaining arterial trauma during and shortly after the Korean conflict.

Material and Methods

Muscle specimens taken from the extremities of 30 soldiers whose major peripheral arteries were damaged acutely form the basis of this report. The arterial injuries comprised severances, lacerations, perforations, and contusions resulting in spasm or thrombosis. In 22 cases, the injuries were due to fragmenting missiles, in 7, to bullets. One soldier sustained vascular trauma in a vehicular accident. The patients ranged in age from 19 to 40, with an average of 24 years.

Samples of skeletal muscle were obtained in three ways: (1) Biopsy. Twenty-five specimens were taken from 19 soldiers during initial débridement of a wound, at the time of fasciotomy, or coincident with revision or closure of a wound. An effort was made to secure tissue for biopsy from regions of muscle which had not been directly traumatized, so that the changes in most of the specimens were attributable to deprivation of blood supply alone. Most of the samples for biopsy were placed in saline solution for 15 to 20 minutes

* Received for publication, October 6, 1955.

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to reduce the incidence of artifacts and were fixed subsequently in 10 per cent formalin. (2) Surgical excision of degenerating muscle groups (3 cases). (3) Amputation (15 cases). In most instances the entire amputated specimen was received by the pathologist. A few specimens were dissected by the surgeon, and formalin-fixed samples of various muscles were forwarded to the laboratory.

The clinical aspects of the patients' injuries were analyzed. The various samples of muscle were embedded in paraffin and stained with hematoxylin and eosin. A bacterial stain and connective tissue stains were done in selected cases. In addition to routine microscopic examination, all stained sections were viewed using crossed Nicol prisms. Chemical determinations of myoglobin and hemoglobin content were done on one specimen.

Review of the Literature

Experimental Studies

Because of the complexity of the human vascular system and the great number of factors involved in clinical cases of vascular injury, most of the fundamental knowledge of muscle ischemia has been gained by carefully executed animal experiments. Attempts have been made by various investigators to characterize the changes in skeletal muscle secondary to (1) obstruction of arterial blood flow, (2) obstruction of both arterial and venous blood flow, and (3) pure venous obstruction.

Obstruction of Arterial Blood Flow

The effects of experimental obstruction of arterial blood flow on muscle depend upon the degree of obstruction, the length of time it has been allowed to exist, and the duration of the recovery period before pathologic examination is done. When the arterial flow is more or less completely interrupted, either by multiple ligations designed to eliminate collateral circulation, or by the application of a tight tourniquet, massive areas of muscle necrosis result.[1] Early in the course of such experiments the ischemic muscles may go into a temporary state of contracture, simulating rigor mortis.[2,3] Eventually, a permanent contracture not unlike Volkmann's contracture in humans may supervene.[1,2,4] In the end stage of severe arterial ischemia, the muscle is hard and is composed of yellow or greenish-yellow infarcts separated by scar tissue.

Following somewhat less than complete interruption of arterial

blood flow, smaller portions of the muscle may undergo infarction.[5,6] Early in the ischemic period the muscle may be pale and swollen with fluid.[5] In small animals such as the rabbit, this degree of ischemia may be followed by more or less complete regeneration and reconstitution of the muscle.[7]

With still less severe grades of ischemia, such as may obtain following ligation of a single main artery of a muscle, weakness[2] or paralysis,[5] from which the animal subsequently recovers, may be the only recognized effect. In some instances of single arterial ligation, the collateral circulation is so efficient that no functional or structural change in the muscle is detected.[5,6]

Harman[8] has described in detail the histologic changes in muscle which has been completely deprived of its arterial blood. The longitudinal fibrils of the fibers become vague and lose their wavy character, while the cross striations grow increasingly more prominent. Eventually, the latter are so accentuated that they appear as broad disks separated by clear spaces (Bowman's discoid degeneration). At intervals, the fibers crack apart between the disks. The fibers meanwhile have become separated from one another, appearing as long individual units. This individualization creates an appearance distinctly different from the "syncytoid" aspect of normal skeletal muscle fibers. A relatively late change is degeneration and eventual disappearance of muscle nuclei. In a later study Harman and Gwinn[9] correlated the histologic findings in ischemic muscle with its contractibility, its content of energy reserves, and its ability to recover. They found that (1) the presence and strength of contraction upon stimulation by faradic current are related directly to the number of histologically undamaged fibers; (2) muscles which are unable to contract and are depleted of their energy reserves recover functionally and resynthesize their energy reserves, provided that they contain a sufficient number of structurally intact fibers. The authors' conclusions suggest that histologic examination provides a more accurate index of viability of muscle than biochemical measurement of energy reserves or contractibility tests.

Clark and Blomfield,[6] who appear to have produced lesser degrees of ischemia than Harman,[8] described microscopic changes which are probably in part due to the retention of a small amount of arterial flow. Such incompletely devascularized muscles showed swelling of fibers with preservation and accentuation of cross striations, weak staining of fibers with eosin, edema and necrosis of the endomysium

(connective tissue framework between individual muscle fibers), exudation of neutrophils between the fibers, and scattered foci of hemorrhage. Later, histiocytes became the principal inflammatory cells as they phagocytized the necrotic muscle; fibroblasts from adjacent, relatively normal muscle grew into the endomysial exudate and formed new endomysial tubes; and muscle nuclei proliferated into the tubes in the wake of digestion of the dead fibers by the histiocytes. The new fibers acquired longitudinal fibrils early in their development; later, cross striations appeared. The initially thin new fibers gradually attained normal thickness. The ultimate result was a completely reconstituted muscle showing variable degrees of fibrosis.

The changes so far described have been those of unrelieved arterial obstruction. Several authors have commented upon striking changes which may occur following restoration of circulation. These include the appearance of tense swelling,[3,5,10] capillary engorgement,[11] edema,[10,11] hemorrhage,[5,10] acute inflammatory exudation,[11] and release of myoglobin.[12] Harman and Gwinn,[9] who produced essentially complete interruption of arterial flow by means of a tourniquet, described a sudden increase in the rate of muscle fiber damage upon release of the tourniquet. They noted the appearance of two new types of muscle degeneration which were not seen in unrelieved total ischemia. These were Zenker's degeneration (in which the fibers became swollen, homogeneous, and structureless) and granular degeneration (in which they became granular, disorganized, and deeply acidophilic). Harman[11] further presented evidence that muscle capillaries and possibly finer arterioles and venules were damaged in ischemia, and that following restoration of circulation, blood flow through them was sluggish. Such vascular damage may account, at least in part, for the severe changes occurring in ischemic muscles upon resumption of their arterial flow.

Obstruction of Both Arterial and Venous Blood Flow

Because of clinical claims made during World War I that simultaneous ligation of veins reduced the incidence of gangrene in limbs requiring arterial ligation,[13,14] several workers have approached the problem of ligation of veins using experimental animals. The results have been conflicting. Brooks, Johnson, and Kirtley[4] concluded that simultaneous ligation lessens the incidence of gangrene and muscle contracture, while Wilson[2] detected no difference in the distribution or extent of muscle necrosis, or in the incidence of contracture.

Pure Venous Obstruction

Brooks[5] and Middleton[15] investigated the effects of obstruction of the entire venous outflow of a muscle whose arterial supply was intact. The muscle became swollen, dark blue, and bloody. Microscopically, there was hemorrhage, necrosis of fibers, and marked neutrophilic infiltration. Later, proliferation of fibroblasts took place between individual muscle fibers. The hemorrhage, inflammation, and fibrosis spread into the surrounding tissue. The end result was a muscle with extensive fibrosis; occasionally, individual muscle fibers were no longer detectable. Contracture was produced constantly. Working with entire limbs instead of isolated muscles, Fontaine and de Sousa-Pereira[16] showed that complete ligation of the venous system was necessary before gangrene could be produced.

It must be kept in mind, in interpreting the results of many of the experiments described, that units of the vascular system other than the ones specifically obstructed can undergo significant secondary changes, and that these may be difficult to recognize and evaluate unless elaborate methods of investigation are used. The stasis which occurs in capillaries secondary to arterial obstruction has already been mentioned. Barnes and Trueta[10] presented evidence that a severe and persistent arterial spasm could be produced in legs by the application of a tight wire tourniquet. This spasm affected main arteries and collaterals as well as, on occasion, the main artery of the opposite uninjured limb. Harman,[11] conducting experiments similar to those of Barnes and Trueta, failed to observe evidence of arterial spasm, and attributed the positive findings of these investigators to the use of a highly irritating radio-opaque medium for determining vessel caliber. On the other hand, the observations of Laufman, Martin, and Tuell[17] supported the concept of the existence of secondary vasospasm in vascular occlusion. These workers measured vessel caliber micrometrically in a series of experimental procedures on the mesenteric vascular tree. They found that: (1) Vasospasm generally accompanies main stem vascular occlusions. (2) In venous occlusion there is a marked arterial spasm and a venous dilatation. (3) Following release of venous occlusion, the artery remains in a state of moderate spasm for a considerable period of time. (4) In arterial occlusion, there is a marked arterial spasm and a concomitant venous spasm. (5) Following release of arterial occlusions, grossly visible reactive hyperemia occurs, but during this state, the precapillary arteriole remains in spasm.

STUDIES IN HUMANS

Very little is recorded of the pathology of skeletal muscle ischemia in man. Military surgeons[18,19] have characterized muscle hours after arterial injury as hard, tense, and swollen. Foisie[20] described the typical appearance of the biceps muscle early in the course of Volkmann's ischemic contracture as swollen, gray, and lifeless.

A major contribution to the *late* pathology of human muscle ischemia is that of Griffiths,[1] who identified the pathologic lesion of Volkmann's contracture as being muscle infarction. The affected muscles are composed of hard, homogeneous yellowish cores, surrounded by scar tissue. Microscopically, masses of necrotic muscle are enclosed successively by zones of histiocytes and fibroblasts, and dense collagen. Griffiths presented convincing evidence confirming Volkmann's concept that the muscle infarction characteristic of his contracture is due to a deprivation of arterial blood. This may occur secondary to embolism as well as to arterial trauma.

Bowden and Gutmann[21] studied muscle specimens obtained for biopsy from 14 patients at intervals of 40 to 800 days after trauma. They attributed three types of pathologic change to ischemia: massive necrosis, diffuse interstitial fibrosis, and focal necrosis with interstitial fibrosis. In all patients with massive muscle necrosis there was evidence of past damage to the main artery of the limb or to the artery supplying the affected muscle or muscles. In patients whose muscles showed the other types of pathologic change there was no uniformity in the nature of the vascular disturbance; in several instances the accumulation of blood in a rigidly bound muscle compartment was implicated; in others, the pathogenesis of the muscle damage was not clear.

Whether pure venous obstruction ever produces serious muscle degeneration in man is disputable. Rare cases of muscle fibrosis and contracture have been reported[15,21,22] wherein the histologic picture has been similar to that of experimental pure venous obstruction.

The significance of venous obstruction when superimposed on arterial blockage in humans is equally controversial. Some surgeons have claimed that a degree of venous obstruction is beneficial in arterial insufficiency.[13,14] On the other hand, DeBakey and Simeone,[19] in reviewing Makins' cases of arterial injury in World War I and the American cases in World War II, concluded that there is no evidence from human experience that venous ligation furnishes protection against the development of gangrene.

Results

Muscle changes during the first 2 days after arterial injury

Thirteen samples of muscle were taken from 11 soldiers at intervals of 6½ to 27 hours after arterial injury.

Clinical Appearance of Muscles

Four soldiers had muscle contractures resembling rigor mortis. The involved muscles were described as being hard, tight, and fixed. In 4 individuals, 2 of whom had contractures, the muscles were considered by the surgeon to be severely ischemic and probably irreversibly damaged. These muscles exhibited one or more of the following: a soft or mushy consistency, a pale or bluish gray color, a failure to bleed or delayed bleeding on incision, and an inability to contract on pinching. The muscles in the remaining 5 cases appeared normal.

Microscopic Appearance of Muscles

Many of the specimens exhibited artifacts or changes more properly attributable to trauma than to ischemia. The more common of these were blurring and patchy fragmentation of cross striations, retraction of fibers from their sarcolemmas and separation from one another, swelling and loss of structure of sarcoplasm, and nuclear shrinkage or swelling. Certain of these changes were considered artifactitious only when they appeared in relation to the cut edges of the specimens.

Findings of questionable etiology, probably related to ischemia in some of the specimens, were congestion of small blood vessels and petechiae. Excluding the presence of these changes, the five samples of muscle considered normal by the surgeon were unremarkable microscopically. Specimens from the three muscles which were in contracture, but which did not appear otherwise abnormal at operation, were likewise unremarkable microscopically.

Of the five specimens of muscle regarded as severely ischemic by the surgeon, two showed a subtle but definite exaggeration of cross striations as the only significant change (Fig. 2). Some of the striations, in addition to appearing coarser than usual, were smoothly curved instead of straight. One "severely ischemic" muscle was remarkable only for the presence of intense neutrophilic infiltration in the walls of its veins. Finally, two muscles placed in the severely damaged category by the surgeon showed marked changes histologically. These comprised, in addition to exaggeration and curving of cross striations, separation and individualization of fibers, and striking

engorgement (thrombosis?) of small vessels with erythrocytes (Fig. 3). The specimen of one "severely damaged" muscle further exhibited focal necrosis of several medium-sized arteries. The necrotic lesions were characterized by fibrinoid degeneration, slight neutrophilic infiltration, and marked edema of the vessel walls; and an infiltration of round cells into the perivascular connective tissue. These changes resembled strongly those seen in polyarteritis nodosa.

MUSCLE CHANGES DURING THE FIRST 2 DAYS AFTER SURGICAL REPAIR OF ARTERIAL INJURY

Five specimens were obtained for biopsy from 5 soldiers at intervals of 24 to 56 hours after arterial injury (and 12 to 48 hours following surgical repair).

Clinical Appearance of Muscles

In 4 of the 5 soldiers a striking clinical feature was the onset of muscle swelling some hours after vascular repair. When fasciotomies were done to relieve the tension within the muscle compartments, the swollen muscles bulged through the incisions. In some instances, blood-tinged fluid exuded. In addition to swelling, all four muscles exhibited changes suggestive of severe ischemia. In one case the muscle was not swollen and appeared normal at operation.

Microscopic Appearance of Muscles

The sample of the grossly normal muscle was unremarkable. The specimens of the four swollen, "severely ischemic" muscles, on the other hand, exhibited diverse and striking changes. In one there was advanced necrosis characterized by disruption and loss of structure of fibers, and extensive neutrophilic infiltration. These changes were far out of proportion to, and different in kind from, those ordinarily observed in purely ischemic muscle, and were interpreted as being secondary to contusion. In the second case, the muscle of the anterior tibial compartment gradually swelled after repair of a lacerated superficial femoral artery. A fasciotomy, done 34 hours postoperatively, revealed swollen muscle which appeared severely ischemic. The specimen taken for biopsy showed changes consistent with early ischemia: exaggeration of cross striations, congested small vessels, and numerous hemorrhages. In the third case, pulsations became palpable in the foot following relief of spasm of the common femoral artery; later they disappeared as the calf muscles began to swell. At the time of fasciotomy, 23 hours after restoration of circulation, the gastrocnemius muscle was swollen and was considered "probably

non-viable." Microscopically (Fig. 4), most of the fibers were closely approximated. Over half of them showed degenerative changes. These included waxy swelling characterized by deep staining of sarcoplasm, nuclear pyknosis, and focal rupture of fibers (Zenker's degeneration); vacuolar degeneration; exaggeration of cross striations; and weak staining with eosin. Many fibers, nevertheless, were normal in appearance. In the last case, the flexor muscles of the arm had been in contracture prior to operation; although warmth was restored to the arm by anastomosis of the severed brachial artery and evacuation of its thrombus, the contracture increased thereafter, and the muscles of both flexor and extensor compartments began to swell. A small specimen of flexor compartment muscle showed slight separation of many fibers, exaggeration and slight curving of cross striations, and small foci of waxy swelling of fibers.

LATER MUSCLE CHANGES (4 TO 26 DAYS AFTER ARTERIAL INJURY)

The pathologic material, obtained from 20 soldiers 4 to 26 days after injury, included five specimens taken for biopsy, three specimens composed of fragments from muscle compartment excisions, six samples taken from amputated limbs, eleven entire amputated limbs, and one amputation stump.

Clinical Appearance of Muscles

Adequately detailed data were unfortunately not available on the clinical appearance of most of the cases. Although, in some instances, the surgeon's observations must have been similar to the gross observations of the pathologist, it is obvious that such properties as color and consistency may often have differed at operation and at the dissecting table. Therefore, the following gross pathologic findings described cannot be regarded as coinciding in every respect with the findings of the surgeon.

Gross and Microscopic Pathology of Muscles

A wide range of pathologic changes characterized the later stages of muscle ischemia. Essentially four types of muscle were seen. Categorized in order of decreasing damage, these were: (1) more or less completely necrotic muscle with little or no evidence of inflammation or repair; (2) muscle showing patchy, and usually extensive necrosis, accompanied by inflammatory and reparative responses; (3) severely damaged muscle with small foci of complete necrosis, but notable for widespread survival of stroma and muscle regeneration; (4) essen-

tially normal or minimally damaged muscle. Although borderline forms existed among these four categories, and although one portion of a given muscle might fit into one category and another portion into a second, by and large an entire muscle or a large part of it was uniform in its pathologic appearance.

1. Complete necrosis was encountered most often in the long slender muscles of the leg. On dissection of the amputated limb these muscles appeared to be of normal color or somewhat pale. Their consistency was normal or slightly flabby. They were at times swollen and bulged slightly when their fasciae were incised. Microscopically, there was extensive necrosis involving both fibers and interstitial tissue. The fibers were closely approximated in some instances (Fig. 5) and widely separated in others (Fig. 6). Although some fibers had structureless sarcoplasm and appeared slightly swollen, the predominant change was one of discoid necrosis. Many of the disks were curved and there was periodic transverse cracking of the fibers between them (Fig. 6). The sarcoplasm often stained feebly with eosin. The nuclei showed increasing degrees of shrinkage and disappeared in time. In the areas of most advanced damage the fibers were split longitudinally and transversely or were fused into coarsely granular amorphous masses (Fig. 5). The interstitial tissue showed necrosis as evidenced by its weak staining and shrinkage of its nuclei. The small vessels were collapsed and their contents were no longer recognizable as blood. There was no congestion; at most, an exceedingly thin peripheral band of neutrophils was the sole evidence of an inflammatory response.

A frequent finding in this type of muscle, if it lay in proximity to a wound or infected incision, was invasion by bacteria, sometimes in massive numbers. Some of these bacteria were morphologically recognizable as Clostridia species. They appeared in the perimysium, endomysium, and within the sarcolemmas. In this small series, gas, edema, or other recognizable lesions attributable to the presence of Clostridia were not seen.

2. Patchy, usually extensive necrosis accompanied by inflammation and repair was observed most often in the soleus muscle; other muscles occasionally showed this change. Grossly, muscle of this type was brownish yellow, or so light yellow that it was easily mistaken for fat. A common appearance was a geographic pattern of pale brown patches of necrosis separated by yellow or white bands of inflammatory exudate. Gross areas of hemorrhage often were visible. Although the consistency generally was about normal, it sometimes was mushy or semi-liquid. Microscopically, there was extensive patchy death of

both fibers and interstitial tissue. The fibers sometimes showed a more or less pure picture of discoid necrosis (Fig. 7); more often, however, there was a considerable admixture of discoid fibers with swollen fibers having blurred or absent striations (Fig. 8). It also was not uncommon to find dead fibers which had retained exceedingly delicate cross striations. The fibers generally were separated from one another, sometimes by empty spaces, at other times by edema fluid and disintegrating neutrophilic exudate (Figs. 7 and 8). When the muscle was liquefied, fragments of disintegrating fibers penetrated by neutrophils might be seen lying in seas of exudate or hemorrhage. The interstitial tissue in the dead areas stained weakly and exhibited pyknosis of its nuclei. The small vessels were either collapsed and necrotic or were distended by closely packed erythrocytes. The larger vessels in and near the zones of necrosis commonly showed inflammation or necrosis of their walls. Often the veins, and less frequently the arteries, were distended by thrombi (Figs. 8 and 9) which eventually underwent organization.

In the muscle peripheral to the areas which had undergone complete necrosis, a variety of changes was seen. Early, bands of disintegrating neutrophils characterized this zone (Figs. 9 and 10). Later, histiocytic invasion of sarcolemmic tubes, with digestion of degenerating sarcoplasm, and the appearance of chronic inflammatory cells were prominent features. Proliferation of capillaries and of fibroblasts laying down collagen and an intense but limited muscle cell regenera-

TABLE I

Pigment Content of Muscle *

	Normal muscle		Yellow muscle	
	First determination	Second determination	First determination	Second determination
Myoglobin	5.12	5.09	2.57	2.52
Hemoglobin	0.27	0.26	0.53	0.50

* Given as per cent of dry weight (12 hours at 120°C.). Modification of method of Björck.[30]

tion were still later activities. We have observed no more than minimal penetration of fibroblasts, capillaries, and regenerating muscle fibers into the areas of completely necrotic muscle.

In one case the myoglobin content of a pale yellow muscle belonging in this category was analyzed, and was found to be half its normal value (Table I).

3. Severely damaged but live muscle showing widespread regen-

erative activity was encountered in 2 cases—in the triceps muscle 8 days after wounding, and in the gastrocnemius muscle 10 days after wounding.

The triceps was cream-colored with focal patches of hemorrhage. The gastrocnemius presented, in large part, a color somewhat paler than that of fat. Both muscles were of more or less normal consistency. Microscopically, there were severe degenerative changes within the fibers, but the interstitial tissues and many muscle nuclei survived. The degenerating sarcoplasm was either discoid, or structureless, or fragmented. The fibers were closely approximated or were separated by an edematous endomysium containing scattered histiocytes and round cells (Figs. 11 and 12). The perimysium was edematous and contained inflammatory cells in small numbers. Within the sarcolemmic tubes were focal collections of histiocytes in the process of digesting the degenerating sarcoplasm (Fig. 11). Along the edges of the fibers, nuclei were missing here and there. Elsewhere, however, were rows of elongated cells with scant cytoplasm (Figs. 12 and 13). Some of these were recognizable as regenerating muscle cells with basophilic cytoplasm; others as the endothelial cells of elongated tubular capillaries (Fig. 13).

What appeared to be later phases of the same process microscopically were observed in two additional specimens 12 and 18 days after wounding. These muscles showed large numbers of thin regenerating fibers arrayed in orderly fashion in very edematous viable collagenous tissue (Figs. 14 and 15). The degenerated fibers were no longer recognizable, and the evidence of previous damage was the presence of variable numbers of lymphocytes, plasma cells, and histiocytes among the new fibers. The latter had longitudinal fibrils; in a few of them cross striations could be identified.

4. Essentially normal muscle. Of the muscles examined microscopically, the gastrocnemius or large portions of it most commonly fell into the category of essentially normal. Muscle of this type sometimes exhibited focal damage to fibers in the form of waxy swelling or vacuolar degeneration. Small areas of inflammation, regeneration, and repair also were seen.

A striking finding in the amputated legs was the relatively better condition of the gastrocnemius than of the soleus muscle after a major artery of supply had been damaged. Thus, of 10 cases in which damage occurred to either the femoral or popliteal artery, and in which sections of both gastrocnemius and soleus muscles were available for study, the former showed a lesser degree of ischemic change in 8 cases. In some instances the gastrocnemius appeared

relatively normal when the soleus was either necrotic or regenerating; in others, the former was regenerating when the latter was necrotic; while in still others, the former, though extensively damaged, exhibited less complete necrosis than the latter. In 2 cases there was little difference in microscopic appearance between the two muscles.

The use of special connective tissue staining afforded additional data on the microscopic characteristics of ischemic muscle. With phosphotungstic acid hematoxylin and Masson's trichrome stains (using aniline blue for the latter), degenerating and necrotic muscle fibers often failed to show normal staining properties. These damaged cells were weakly colored or exhibited atypical colors, such as blue (with Masson's) or buff (with the phosphotungstic acid hematoxylin stain, Fig. 7). Waxy fibers, swollen necrotic fibers, discoid fibers, and the degenerating sarcoplasm of the muscles of category 3 commonly showed weak or atypical staining. Regenerating muscle cells stained normally with the connective tissue stains in our small experience.

Using crossed Nicol prisms, it was found that muscle fibers commonly retained their birefringence in advanced stages of necrosis. Indeed, swollen fibers and fibers showing discoid necrosis were often brightly refractile even after their nuclei had completely disappeared. Refractility was lost in some fibers showing advanced discoid or structureless necrosis and in fibers exhibiting granular disintegration. It reappeared in early regenerating fibers.

Discussion

Since most of our basic knowledge of ischemia of skeletal muscle has come as a result of animal experimentation, a comparison of clinical and pathologic observations in humans with experimental findings is in order.

Contracture

An inconstant early manifestation of muscle ischemia in humans is contracture. This phenomenon is not to be identified with the permanent Volkmann's contracture, although it is possible that the former represents an initial, reversible stage in the development of the latter. In this series, no instances of permanent contracture were encountered. No structural basis for early ischemic contracture was discovered in the human specimens. In the earlier cases, in which the muscles involved appeared otherwise normal at operation, microscopic examination of the samples was unremarkable. In later cases, in which the muscles exhibited other evidences of ischemia at surgery, the specimens taken for biopsy showed changes similar to those seen

in ischemia without accompanying contracture. Early ischemic contracture has been produced experimentally in muscles in which the arterial blood supply has been more or less completely interrupted[2,3]; however, its nature has not been investigated further.

Histopathologic Features

The earlier structural manifestations of muscle ischemia in humans were similar to those described by Harman[8] in experimental animals (loss of wavy arrangement of longitudinal fibrils, exaggeration of cross striations, and separation and individualization of fibers). Straightening of the longitudinal fibrils, however, was not a reliable sign of early ischemia in man, for, although it appeared in all of the "severely ischemic" specimens, it was seen in several samples of otherwise normal muscle as well. This discrepancy may be due to the fact that the uniform fixation and staining possible in experimental material was not attained in our specimens. The vascular engorgement (thrombosis?) observed in two of the human "severely ischemic" muscles may have been the structural counterpart of the physiologic damage to small vessels demonstrated by Harman[11] in experimental muscle ischemia. The unusual vascular changes seen in two of the human specimens, i.e., the neutrophilic infiltration of the vein walls, and the necrotic arterial lesions resembling those of polyarteritis, were unique and have not, to our knowledge, been described in experimental ischemia. Interpretation of their relationship to ischemia must remain conjectural at the present time.

Swelling

Swelling of ischemic muscles occurs in humans with arterial injury as well as in experimental animals. In humans it may take place in instances of unrelieved ischemia; however, it is more frequent and more severe following surgical restoration of circulation. In experimental animals, ischemic swelling may appear as a result of less than complete interruption of arterial blood[5] or may follow release of arterial obstruction.[3,5,10] Neither animal investigation nor biopsy examination of human swollen muscle has revealed the pathogenesis of ischemic swelling. It seems possible that five factors, alone or in combination, may contribute: (1) lymphatic stasis, (2) vascular congestion, (3) enlargement of individual fibers, (4) interfibrillar edema, and (5) edema of the perimysium (connective tissue between bundles of fibers). Our specimens taken for biopsy did not permit evaluation

of the first and fifth factors; and none of the other factors was encountered with regularity. The presence of Zenker's degeneration in two human specimens of muscle swollen hours after restoration of circulation is most interesting in view of the fact that Harman and Gwinn[9] found this change under similar circumstances in their experimental animals. In the small biopsy specimen of one human muscle, the change was present in only a few foci; in the other, it was widespread. Interpretation of the lesion in the latter case, however, was complicated by the fact that the soldier had been exposed to cold. Since fiber degeneration similar to Zenker's has been reported in experimental hypothermia[23] as well as in relieved ischemia, cold injury cannot be ruled out entirely as the etiologic factor here. If, however, these unusual changes in the two human cases are due to restoration of circulation, Harman's observations on the increase in muscle fiber damage wrought by a return of circulating blood may have a direct application to vascular repair in humans.

Regeneration

Wide varieties of pathologic changes, ranging from minimal damage to massive necrosis, characterize the later stages of muscle ischemia, both clinical and experimental. For the most part, the findings in man and animal have been similar. Two aspects in which clinical and experimental observations are somewhat at variance, namely, regeneration and depigmentation, deserve special attention.

Clark[7] described extensive muscle regeneration and reconstitution in small experimental animals even when the degree of arterial ischemia had been so great that necrosis of the interstitial tissue had taken place. The same author, however, is cited[24] as saying that a similar degree of regeneration would appear unlikely in man because of the bulk of human muscles. We have seen only abortive attempts at regeneration when severe necrosis involving the interstitial tissue as well as fibers has occurred. However, several of our specimens in which the sarcoplasm had undergone widespread degenerative changes, but in which the stroma had survived, showed such striking early regeneration that a considerable amount of eventual reconstitution seemed possible. The pathologic changes in such muscles were more akin to those of Zenker's degeneration of typhoid fever and pneumonia[25] than they were to those of the more severe degrees of ischemia. More extensive experience with human cases of ischemia is needed to confirm and expand these observations.

Depigmentation

Although Montagnani and Simeone[12] demonstrated liberation of myoglobin from muscles upon release of ischemia, visible depigmentation as a phenomenon of experimental muscle ischemia has not, to our knowledge, been described. Our studies have shown that in humans severe and widespread loss of pigment may occur not only in muscles which show large areas of necrosis, but also in those exhibiting regenerative changes. Thus, the appearance of a fish-flesh, cream, or pale yellow color in a muscle does not necessarily indicate that it is irreversibly degenerated. In our brief experience, muscle which is more or less completely deprived of its blood supply does not undergo depigmentation, perhaps for the reason that no circulatory system exists in the muscle to furnish enzymes necessary for the release of the myoglobin and to provide for transportation of this pigment from the muscle.

Pathogenesis of Ischemia

Whereas it is difficult to determine in experimental animals what pathogenic factors are responsible for producing the various forms and degrees of ischemic degeneration of muscle, this is an even greater problem when dealing with human cases. Here the variables are multiplied many fold and investigative methods are necessarily limited. Thus, in our series, often only a biopsy specimen was available for study. This may not always have been representative of an entire muscle, nor did it afford information about the state of the blood vessels supplying the muscle. In cases in which an entire amputated limb was forwarded for examination, usually the key obstructed vessel remained in the patient above the amputation site. Spasm of arteries or veins and the degree of obliteration of collateral vessels were most difficult, if not impossible, to evaluate. Many of the soldiers had had tourniquets applied; although in most cases information was given as to the duration and periods of release, it was not possible to determine how effectively the arterial flow had been obstructed. The degree of shock, exposure to excessive cold or heat, the state of fatigue of the muscle at the time of onset of ischemia, and individual variations in vascular supply were some of the other factors which did not lend themselves to accurate determination.

In view of the foregoing, we are unable to state the precise rôles of arterial, venous, and capillary obstruction in producing the muscle changes observed. In addition to arterial damage, large veins were injured directly or were ligated in many of our cases. Moreover,

major and intramuscular veins, and capillaries were often extensively thrombosed in the pathologic specimens. In some instances the thrombi formed in vessels damaged by the same ischemic infarction which injured the muscles they drained.

There is a remarkable similarity between the pathologic changes of muscle ischemia secondary to arterial trauma and those of the crush syndrome. In the latter condition, muscle swelling, discoid necrosis, atypical staining of fibers with connective tissue stains, and regeneration have all been described.[26] Moreover, gross depigmentation of muscle similar to that of the crush syndrome and even renal failure may be seen in cases of arterial injury without crushing.[27] The striking pathologic resemblance of the two conditions supports other evidence that the muscle changes of the crush syndrome are due to arterial spasm.[27,28]

A final observation that merits brief discussion is the usually better outcome for the gastrocnemius than for the soleus muscle in injuries of the femoral or popliteal arteries. The reason for this finding is not apparent from this study, nor from the literature. Blomfield,[29] who has investigated the blood supply of the leg muscles by injection techniques at necropsy, has stated that the gastrocnemius is served by a single artery; the soleus, on the other hand, has at least five arteries of supply. He stated further, that in local wounds of the calf, the gastrocnemius is more apt to undergo necrosis and secondary clostridial infection because of its less rich blood supply. The disparity between the outcome of the two muscles following local vascular damage and following injury to a major artery of the extremity deserves investigation. Possibly the artery supplying the gastrocnemius communicates by collateral channels with vessels arising above the level of obstruction of the femoral or popliteal artery. Again, the comparative tightness of the sheaths or fascial envelopes of the two muscles, or a difference in their metabolic requirements may play a decisive rôle in their responses to ischemia. A practical corollary of the observation regarding the gastrocnemius and soleus is that the condition of the former muscle cannot be used as a guide to that of other muscles of the leg.

Summary and Conclusions

The early pathologic changes in skeletal muscle following damage to major arteries of limbs were studied in 30 soldiers, most of whom had sustained injuries on the Korean battlefields. The material consisted of biopsy specimens, surgically excised necrotic muscles, and

amputated limbs. The specimens were obtained from 6½ hours to 26 days after injury.

The pathologic lesions observed paralleled those described in experimental muscle ischemia and appeared to be the logical forerunners of later changes reported in humans.

Swelling of ischemic muscles following restoration of circulation occurred in several cases. The nature and pathogenesis of this swelling was not evident from study of human or experimental data.

After the acute phase of ischemia had passed, the muscles studied fell into one of four pathologic categories: (1) more or less complete necrosis; (2) patchy, but extensive necrosis with inflammation and repair; (3) severe damage of fibers, but survival of stroma and widespread muscle regeneration; and (4) normal structure.

A pale yellow or cream color, probably attributable to loss of myoglobin, was an outstanding feature of several muscles in category 3 as well as in category 2. Therefore, depigmentation is not a reliable criterion of irreversible damage in ischemic muscle.

The gastrocnemius muscle fared better than the soleus in the great majority of cases of obstruction of a major artery of the leg. This result was the opposite of that reported in local wounds involving the immediate arteries of supply of the muscles. The vascular anatomical background for this disparity was not clear. Because of the difference in outcome of the two muscles, it is apparent that the condition of the gastrocnemius cannot be used as a guide to that of other muscles of the leg.

We wish to express our gratitude to 1st Lt. Gordon D. LaZerte, M.C., who participated in the early phases of the pathologic study; to Lt. Col. Tomas C. Jefferis, M.S.C., U.S.A., and Cpl. Donald D. Maheu, who performed the myoglobin and hemoglobin assays; and to Sfc. Kaye Ihara, who took the photomicrographs.

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LEGENDS FOR FIGURES

FIG. 1. Histologically normal muscle. The close approximation of fibers and the wavy character of longitudinal fibrils may be noted. Hematoxylin and eosin stain. × 100.

FIG. 2. Exaggeration of cross striations. (For pictorial purposes, a filter was used to emphasize the striations.) Hematoxylin and eosin stain. × 200.

FIG. 3. Separation and individualization of fibers; engorgement (thrombosis?) of small vessel. Hematoxylin and eosin stain. × 100.

FIG. 4. Focal waxy and vacuolar degeneration of gastrocnemius muscle. Many of the fibers appear normal. Hematoxylin and eosin stain. × 100.

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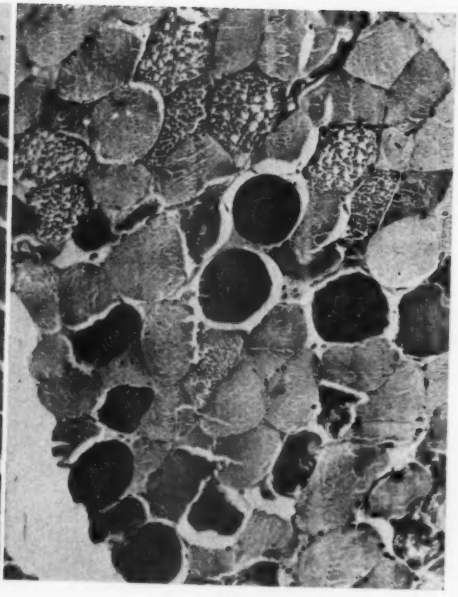

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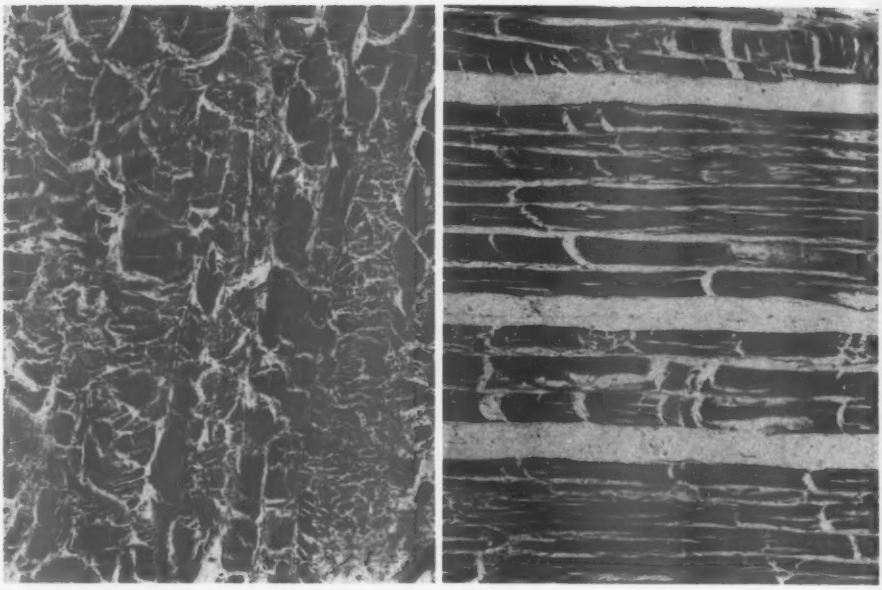

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FIG. 5. Flexor hallucis longus muscle. Fragmentation and granular disintegration of fibers with minimal separation. Hematoxylin and eosin stain. × 100.

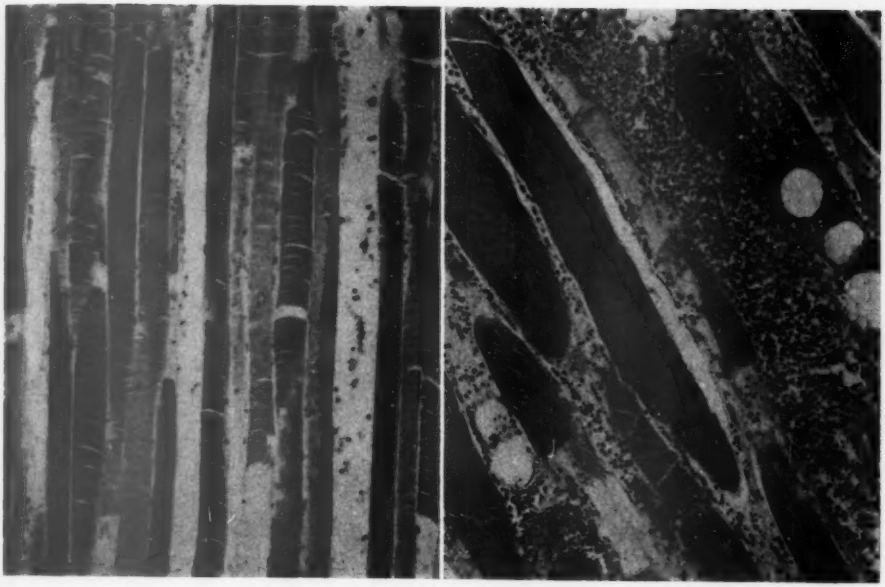
FIG. 6. Extensor hallucis longus muscle. Discoid degeneration with curving of disks and cracking between them. Nuclei have disappeared. Fibers have separated. Hematoxylin and eosin stain. × 100.

FIG. 7. Discoid necrosis of muscle of flexor compartment of arm. Many of the fibers are pale (actually buff) instead of having the normal dark tone (blue). The fibers are individualized and show curving of the disks and cracking between them. Phosphotungstic acid hematoxylin stain. × 100.

FIG. 8. Soleus muscle. Swollen, structureless, anuclear, necrotic fibers are separated by dense neutrophilic exudate. Of note is a thrombosed vein. Hematoxylin and eosin stain. × 100.

FIG. 9. Soleus muscle, with dead muscle above. Below there is extensive neutrophilic infiltration of dead muscle. A large thrombosed vein is visible at the lower right of the center. Hematoxylin and eosin stain. × 35.

FIG. 10. Lateral head of gastrocnemius muscle, corresponding to an opaque brownish yellow area grossly. Of note are discoid necrosis and separation of fibers, and a broad zone of neutrophilic infiltration in the perimysium. Hematoxylin and eosin stain. × 100.



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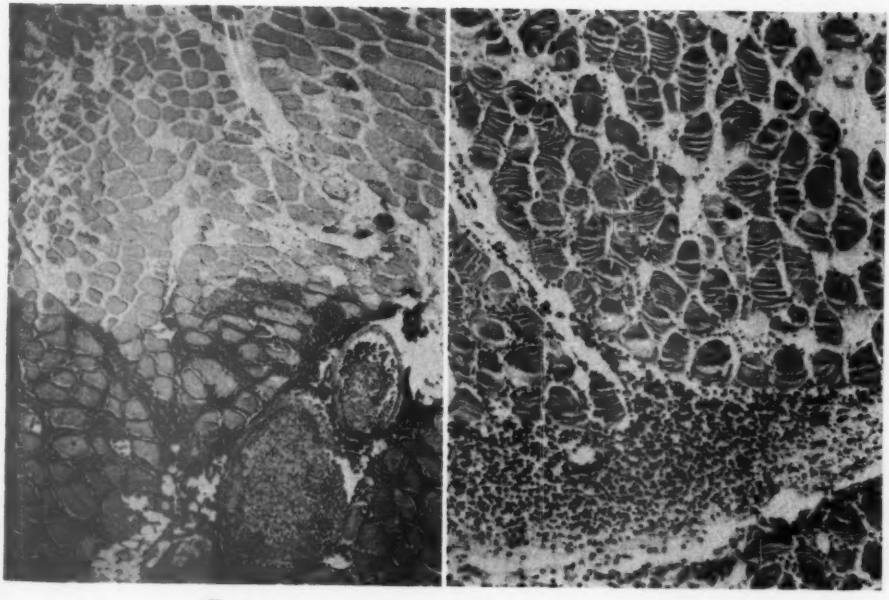

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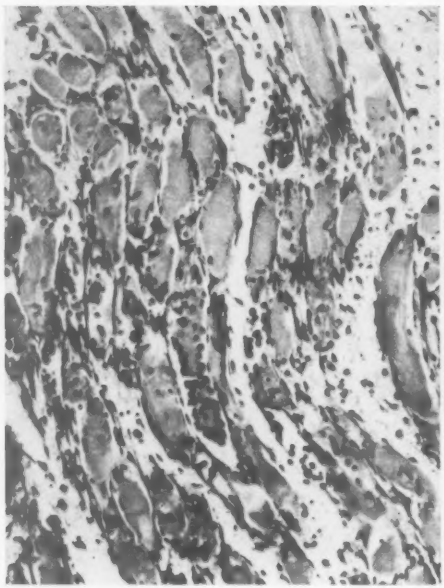

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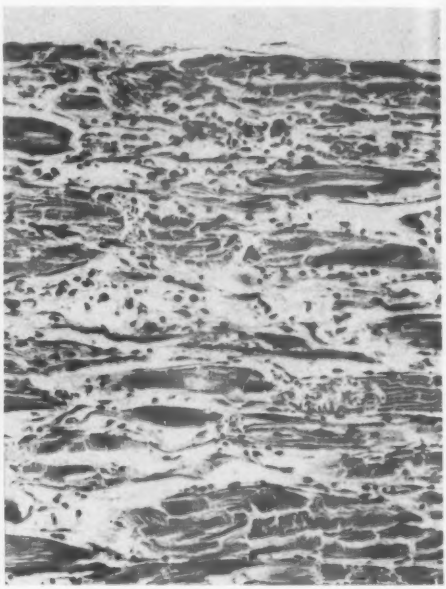

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FIG. 11. Triceps muscle. Fibers show degenerating sarcoplasm. Some sarcolemmic tubes contain large numbers of histiocytes. Most of the fibers are surrounded by necklaces of elongated cells. Hematoxylin and eosin stain. × 100.

FIG. 12. Lateral head of gastrocnemius muscle, corresponding to a pale yellow area grossly. There are elongated bands of regenerating cells among degenerating fibers. Hematoxylin and eosin stain. × 100.

FIG. 13. Lateral head of gastrocnemius muscle. Disintegrating sarcoplasm and elongated capillary tubes are shown. A regenerating multinucleated muscle cell is present near the lower margin. Hematoxylin and eosin stain. × 400.

FIG. 14. Gastrocnemius muscle. Elongated regenerating fibers with multiple clusters of nuclei are separated by histiocytes, chronic inflammatory cells, and edema fluid. Hematoxylin and eosin stain. × 100.

FIG. 15. Soleus muscle. Above are some remaining normal fibers. Below are numerous small regenerating fibers arrayed in an orderly fashion in edematous connective tissue. Small numbers of chronic inflammatory cells lie among the small fibers. Hematoxylin and eosin stain. × 100.

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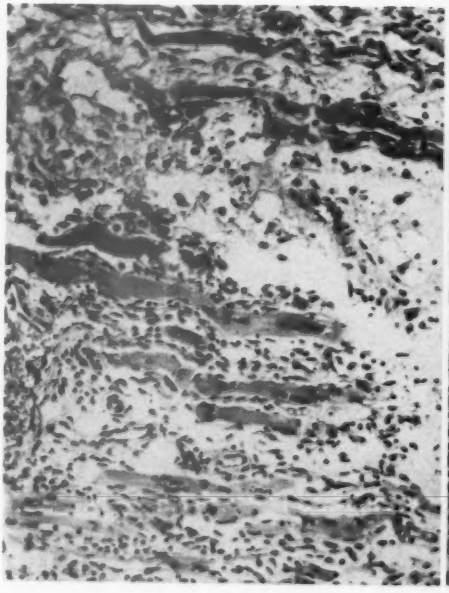

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CHYME EMBOLI IN THE LUNGS OF GOATS WOUNDED BY MISSILES *

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Emboli made up of a wide variety of materials have been reported in the lungs and other organs. The most extensively studied, and probably the more common, are those composed of clotted blood (dislodged thrombi), fat, or air. Pulmonary emboli consisting of single cells or bits of tissue from various organs have been observed after physical trauma and in other circumstances. Various foreign bodies have been found acting as emboli, both spontaneously and after deliberate injection into the vascular system.

A discussion of the pathology of pulmonary embolism resulting from many kinds of emboli is given by Ceelen,[1] with an extensive bibliography through 1930. More recently, as an additional type, amniotic fluid embolism has been described by Steiner and Lushbaugh[2] and by other observers. The present communication reports the occurrence of emboli composed of gastro-intestinal contents in goats, following missile wounds. For brevity, the contents of the rumen and other components of the gastric complex and of the intestinal tract will often be called chyme, although this term (originally meaning juice) does not describe too aptly the partly digested vegetable material normally found in the gastro-intestinal tracts of ruminants. It appears that such chyme emboli have not been observed in man. It is interesting to note, however, that Zenker,[3] in 1862, in reporting one of the first recorded cases of fat embolism, considered the fat he found in the pulmonary vessels to have come from the cavity of the stomach, an opinion apparently not concurred in by later commentators.

Material

The three cases here reported were encountered in the pathologic study of goats used in experiments on the wound ballistics of small missiles, conducted by the Biophysics Division of the Chemical Corps Medical Laboratories. The missiles were fired under controlled conditions. No deliberate attempt was made to produce chyme emboli, these lesions being discovered in the course of routine pathologic examinations. The animals were grade goats procured from the eastern

* Cleared for publication by the Office of Public Information, Department of Defense.
Received for publication, October 26, 1955.

part of the United States. They were in good condition before being shot, as judged by general observation.

Protocols of Observations

The necropsy observations will be described in some detail for goat 1; for goats 2 and 3 only the more pertinent findings will be mentioned.

Goat 1. Necropsy no. G588

This was a well developed male goat weighing 56 kg. All incisors were of the permanent type (indicating an age of at least 4½ years). The animal died 3½ minutes after being wounded, and the necropsy was begun 45 minutes later.

Gross Examination

There was a single wound, produced by a missile passing in an anteroposterior direction. The skin wound of entrance was on the ventral surface of the midportion of the neck, very near the midline. The bullet tract passed through the ventral wall of the underlying trachea. The missile must have traveled for some distance within the tracheal lumen, since a wound tract next became apparent in the wall of the right bronchus just beyond the bifurcation of the trachea. The tract continued through several centimeters of lung tissue, burrowing under the base of the mediastinal lobe, with exit from the lung on the diaphragmatic surface of the right diaphragmatic lobe. There followed a through-and-through wound of the esophagus and a wound through the diaphragm at a point 2.5 cm. to the right of the midline. Within the abdomen, there was a large wound of the liver near its anterior edge, with extensive laceration of the organ, particularly on its visceral surface. Here there was a region of maceration and avulsion of liver tissue measuring 8 by 6 cm., with wide lacerations radiating from it for distances up to 6 cm. into adjacent portions of the organ. In the depths of this large ragged wound the hepatic vein was visible and was seen to be severely lacerated near its junction with the vena cava. The vena cava itself was intact, as were all other grossly delineable blood vessels in the abdominal region.

Beyond the liver there was a large tangential wound of the reticulum, producing a gaping defect 7 cm. in diameter in the reticulum wall. Just beyond this wound was one of equal size in the anterior wall of the omasum. Only small amounts of rumen contents were found free in the peritoneal cavity, but the slightest manipulation caused large quantities to exude through the large rent in the reticu-

lum. Within the peritoneal cavity there were present 800 cc. of blood clot and 450 cc. of fluid blood, the bulk of the bleeding clearly having occurred from the lacerated hepatic vein.

The pharynx, larynx, trachea, and bronchi contained large amounts of soft blood clot. In the right pleural cavity there were 600 cc. of clotted and 150 cc. of fluid blood; the left pleural cavity contained only the usual few cubic centimeters of clear fluid. The lungs were bulky and rather soggy, but were crepitant and floated on water. The only direct wound of the lungs was that of the right lung already mentioned. The wound tract here was surrounded by a zone of deep hemorrhagic discoloration several centimeters wide. There were several contusions presenting a similar appearance, but not associated with a wound tract, in the medial portions of both lungs. Such contusions are commonly seen in tissues near the line of flight of a missile and are believed to result from the violent lateral displacement of material during the few milliseconds just after the passage of the missile ("temporary cavity effect").[4] The cut surfaces of the lungs at many other places showed the fine red stippling or leopard-skin appearance characteristic of blood aspiration. Slight leakage of rumen contents into the posterior dorsal mediastinum had occurred through the wound of the esophagus.

Microscopic Examination

Sections through the wounds of the various organs showed the pictures to be expected, in each case, in an animal surviving for only a few minutes, there being some local laceration and disorganization of structure, with surrounding fresh interstitial hemorrhage, but no discernible cytologic changes. In the lungs, besides the picture of recent aspiration of blood, there was a small isolated patch of rather fresh pneumonia. The parenchyma of the pancreas was normal, but the loose connective tissue around it, as well as some of the interlobular septa, contained particles of vegetable material (rumen contents) which appeared to have been driven into these tissues rather than merely to have adhered to them. There were a few minute fresh hemorrhages into the adrenal medullae.

Throughout both lungs, the branches of the pulmonary artery presented a striking picture, practically all of them being dilated and engorged. Within the lumina of many of these vessels there were present, in addition to blood, varying amounts of unmistakable vegetable material, usually in small chunks (Fig. 1). This material resembled very closely the normal contents of the rumen of the goat. In

some instances, the lumen of the artery was almost completely occluded by such material, several of the larger arteries containing veritable miniature "log jams" (Fig. 2). Such chyme emboli were found in arterial branches with diameters at least as small as 0.1 mm.

The vegetable material apparently had been "aspirated" into the abdominal vena cava through the large wound of the hepatic vein, and then swept into the pulmonary arterial tree after passing through the right side of the heart. Additional evidence of this course was available in sections showing blood clot clinging to the endocardium of the right ventricle. Mixed with this clot were small bits of vegetable tissue similar to that in the lung emboli (Fig. 3). Even more decisive was the presence in this clot of several protozoa of the kind normally found in great numbers in the rumen contents (Fig. 4).

Apparently some of the vegetable material had even been carried through the pulmonary circulatory system. In one of the sections from the heart there was an artery containing a narrow strip of plant tissue (Fig. 5). This is not too surprising, since in the goat's lungs there are arteriovenous anastomoses similar to those described in man.[5] Chyme emboli were not seen in any other organs.

Goat 2. Necropsy no. G604

The body was that of a female goat weighing 51 kg. The incisors were of the permanent type and were badly broken and worn down. Death occurred 4 minutes after wounding; necropsy was started 5 hours later.

Gross Examination

There was a single wound, the tract running in an anteroposterior direction through the chest and abdomen. The tract passed through the chest wall at the level of the fifth interspace, 3 cm. to the right of the ventral midline, nicking the sixth rib. There was a wound of the diaphragmatic lobe of the right lung, traversing 13 cm. of lung tissue. The right pleural cavity contained 500 cc. of fluid and clotted blood; the left pleural cavity, only the usual few cc. of clear fluid.

There had been extensive trauma within the abdomen. The peritoneal cavity contained 4,000 cc. of turbid, bloody fluid, and 300 cc. of clotted blood, mixed with rather dry rumen contents. There was a tremendous wound of the dorsal sac of the rumen, 15 cm. in diameter. No other wounds of the gastro-intestinal tract were present. The source of the extensive hemorrhage was apparent. In the left lateral wall of the vena cava, just anterior to the point at which it becomes partly embedded in the liver, there was a large gaping lacera-

tion, measuring 15 by 8 mm. The only other gross vascular wound was a tear, measuring 6 by 4 mm., in the external iliac artery, deep in the pelvis. The dorsal surface of the left kidney had suffered a large but superficial laceration. No other abdominal organs were wounded.

Microscopic Examination

There was patchy medial sclerosis of the aorta. The pancreas showed extensive fresh hemorrhage into the interlobular septa, sometimes extending into the lobules themselves, but no evidence of direct wounding. In the rumen and kidney the expected fresh traumatic changes were noted.

In several of the lung sections there were seen multiple emboli composed of rumen contents. In the larger arteries so affected, such material was mixed with blood (Fig. 6). In some of the smaller arteries the lumen was completely filled with vegetable material. In one section, there was an isolated patch of lung tissue which appeared to have undergone rapid post-mortem digestion.

Goat 3. Necropsy no. G718

This animal was a male goat weighing 42 kg. All incisors were permanent. Externally there were noted 24 wounds, scattered over the right side of the body. The animal died 3 minutes after wounding and was necropsied 2½ hours later.

Gross Examination

There were several small wounds of the lungs. The right pleural cavity contained 100 cc. of fluid and clotted blood. In the peritoneal cavity there were 175 cc. of free blood, and there was diffuse soiling of the peritoneum with the contents of the rumen and colon. There were four wounds through the wall of the rumen, permitting easy escape of the contents, and a wound of the duodenum. There were several very large wounds of the distal part of the colon, ranging up to 15 mm. in diameter. The lumen of the colon proximal to these wounds was full of blood clot, 160 cc. of clot being recovered. The liver was wounded in two places and the right kidney in two places. There was also retroperitoneal hemorrhage, amounting to 100 cc. on the right side and 60 cc. on the left. The vena cava was severely lacerated at a level just posterior to the mouths of the renal veins. The gaping defect in the wall of the vena cava measured 20 by 12 mm. In addition, there was a through-and-through wound of the aorta at a level slightly anterior to the wound of the vena cava, with large

open holes in the aortic wall. The wounds of the vena cava and of the colon were rather close to one another, and a big rent in the peritoneum in this vicinity was so placed as to channel some of the blood from the torn vena cava through the defects in the colon into its lumen.

Microscopic Examination

The wounds of the various organs had the anticipated microscopic appearance. The adrenal cortices contained a few very small fresh hemorrhages. Many branches of the pulmonary artery throughout the lungs contained well defined balls or skeins of fibrin devoid of red cells (Fig. 7). These structures, which were lightly sprinkled with leukocytes, usually lay quite free in the arterial lumen, and never appeared to be firmly attached to the intima. The intima itself was not altered.

Two small arteries were found, after some search, which were occluded by masses of vegetable material (Fig. 8). In the present case, this material was believed to be contents of the colon rather than of the rumen. The chunks of plant tissue were surrounded by an incomplete, narrow layer of loose fibrin, containing many polymorphonuclear leukocytes. In another section of lung, a small artery was plugged by a mass of renal tissue, consisting of a cluster of typical proximal convoluted tubules.

Discussion

The three instances of pulmonary chyme emboli described were the only ones encountered among some 700 necropsies performed in this laboratory through August, 1954, on goats subjected to wounding by missiles. All three showed the same general pattern of wounding, there being in each instance a large wound of the gastro-intestinal tract permitting free escape of material from its lumen, in close proximity to an extensive laceration of the vena cava or one of its major branches. Our experience to date suggests that the factors just mentioned are essential for the production of chyme emboli of the lungs as a result of wounding by small missiles. Death occurred very promptly in all three goats from rapid massive hemorrhage.

The slides from all other animals necropsied before September, 1954, and which had wounds of the gastro-intestinal tract (a total of 135), have been reviewed and no additional instance of chyme embolism has been found. Some of these animals also had wounds of major abdominal veins, but in none were the wounds of the intestine

and the vein as large or as close together as in the three cases showing chyme emboli. A smaller series of consecutive necropsies also has been re-examined, without regard to the nature of the wounds, and again no additional cases of chyme embolism were discovered.

The mechanism of pulmonary chyme embolism deserves some discussion. The sequence of events thought to be operative in many cases of parenchymatous embolism by Young and Griffith,[6] on the basis of their apposite and stimulating experiments with a mechanical model, certainly must be considered. The state of affairs in the present examples, however, does not resemble too closely that prevailing in their experiments. The large veins wounded were not completely surrounded by the contents of rumen or colon, at least as judged from a reconstruction of the situation during the short survival period, as based upon the gross necropsy findings. In each case there was rapid and extensive bleeding from the lacerated veins, in such quantities as to make it very likely that the hemorrhage was continuous rather than intermittent.

It is believed that a mechanically simpler explanation is adequate in these cases with severe traumatic lesions and rapid demise. During the very short period of wound formation (at most, a few milliseconds), it seems likely that contents of rumen or colon were carried bodily into the lumina of the lacerated veins and began to be swept toward the heart before any significant bleeding occurred through the rents in the walls of the veins. On this basis, no chymous material would have been "aspirated" after the very short period of actual wounding, the emboli found in the lungs representing portions of the mass of material forcibly injected during and just after the passage of the missile. In experiments with gelatin tissue models and with animals, Dziemian and Herget[7] have demonstrated that not only does there develop a large, short-lived temporary cavity, but that particulate matter can be drawn into this temporary cavity from the surroundings during the few milliseconds before its collapse. In our experience it is not unusual to find contents of the rumen or colon that have escaped through intestinal wounds, or bits of material from wounded organs, in wound tracts, both proximal and distal to the wounded organs. Thus it is not surprising that such material is sometimes "blown" into the lumina of large veins through tears in their walls.

It seems likely that chyme emboli can occur in man when there are traumatic lesions comparable in type and severity to those de-

scribed in our experimental animals. As already noted, Zenker considered the fat emboli he found in a case of severe crushing injury to the trunk, with almost instantaneous death, to have come from liberated gastric contents. Referring to the severe lacerations of the liver and the complete transecting laceration of the pylorus found at gross necropsy in that case, he wrote[3]:

"Nach diesem Befund lässt sich annehmen, dass bei der gewaltsamen Zerreissung etwas von dem Mageninhalte in die weit klaffenden Mündungen der durchrissenen Lebervenenäste hineingeschleudert wurde, oder bei dem Hinstürzen des Verletzten geradezu hineinfloss, von hier aber mit dem aus der unversehrten unteren Hohlvene noch zuströmenden Blute in's rechte Herz geführt wurde, von wo es die wenigen noch stattfindenden Herzcontractionen bis in die Lungencapillaren trieben." [In view of these findings, it may be supposed that, with the violent tearing, some gastric contents were flung into the widely gaping openings of the lacerated branches of the hepatic vein, or flowed into the veins just after the impact, and from there were carried by the blood still flowing in the inferior vena cava into the right heart, from where the last few agonal heart beats drove the material as far as the lung capillaries.]

Chyme emboli might well be rarer and less extensive in humans than in goats, since man has considerably less capacious food reservoirs. (In the goat some 10 to 15 per cent of the body weight is accounted for by the contents of the rumen and other parts of the gastro-intestinal tract.) Such emboli in man would probably be harder to recognize, since well preserved vegetable tissue is present in much greater volume in the gastro-intestinal contents of the goat. Intravascular fibrin masses like those noted in case 3, presumably resulting from entry of intestinal fluids into the blood stream, might be expected in human cases. Somewhat similar formations have been observed in cases of embolism of amniotic fluid.[8]

Pulmonary emboli made up of vegetable material have been reported by Lovitt,[9] in a case of abortion induced by a douche containing powdered household mustard. The microscopic picture produced in the lung resembled that found in our cases. In Lovitt's case, of course, the plant tissue gained access to the blood stream from the cavity of the uterus, rather than from the lumen of the gastro-intestinal tract.

Summary

There are reported three examples of pulmonary emboli made up of vegetable material from the gastro-intestinal tracts of goats wounded by missiles. In all instances there were present extensive wounds of the rumen or colon, with adjacent large lacerations of major veins.

The mechanism of entry of chymous material into the blood stream in cases of this sort is discussed, especially with reference to the physical events known to occur during the act of wounding by missiles.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

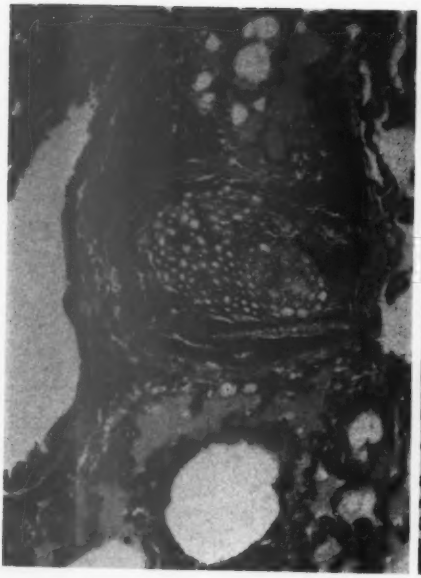
All sections were stained with hematoxylin and eosin.

FIG. 1. Case 1. Chunks of vegetable material in pulmonary artery branch. × 144.

FIG. 2. Case 1. "Log jam" in larger artery. × 50.

FIG. 3. Case 1. Bits of plant tissue in clot from right ventricle. × 144.

FIG. 4. Case 1. Rumen protozoon in clot from right ventricle. × 650.



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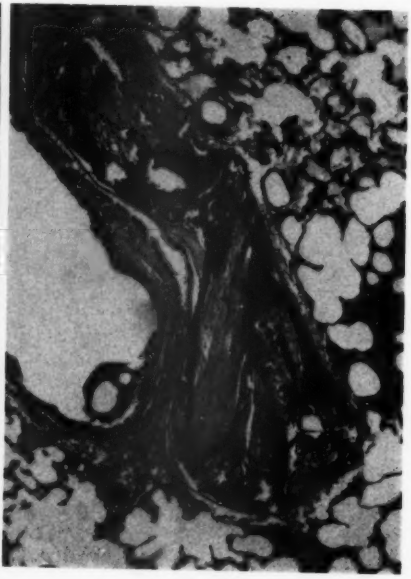

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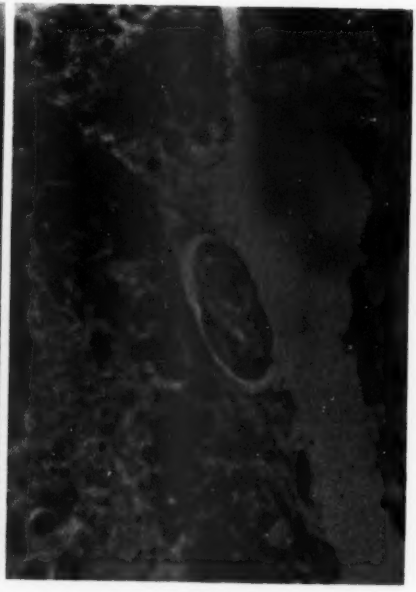

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FIG. 5. Case 1. Vegetable tissue in coronary artery. × 144.

FIG. 6. Case 2. Chyme embolus of lung. × 31.

FIG. 7. Case 3. Portion of fibrin ball in large branch of pulmonary artery. × 144.

FIG. 8. Case 3. A chyme embolus in a small artery. × 406.

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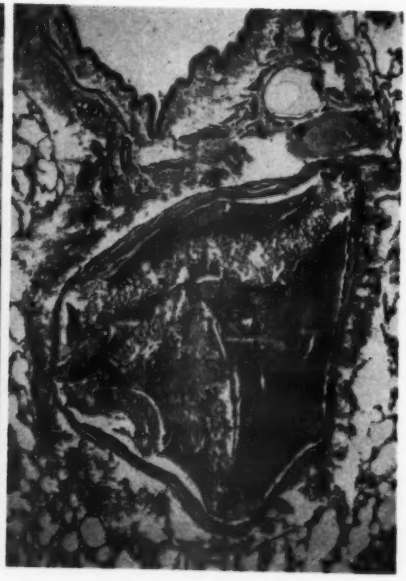

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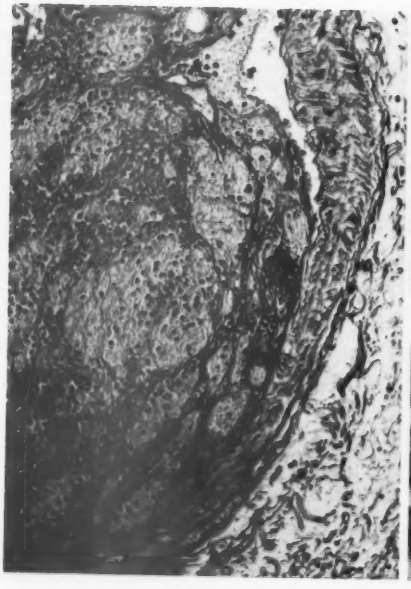

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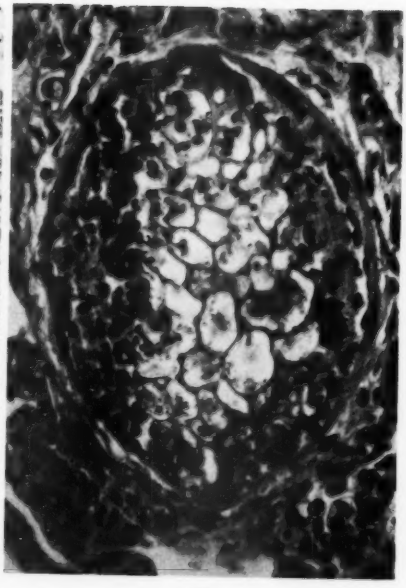

8

HISTOPATHOLOGY OF KIDNEY DISEASE IN FISH *

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Kidney disease is one of the most puzzling fish diseases known to exist in the United States. In less than 10 years it has invaded the Pacific Northwest, exacting a heavy toll of hatchery salmon. Its first appearance apparently was in Massachusetts where Belding and Merrill[1] described a disease similar to that now seen on the Pacific Coast. In 1946 it was diagnosed in Washington[2] and since that time has been observed in an ever increasing number of hatcheries. There are unpublished reports of the same or similar diseases in both California and Washington in the early 1930's.[3] The latest outbreaks occurred in the Federal hatcheries at Berlin, New Hampshire, and Cortland, New York, in brook, brown, and rainbow trout.[4] There is evidence to indicate that the disease may be much more widely spread in New York State.[5]

The disease is especially dangerous since little is known of the origin or source of the causative agent. Indeed, the classification of the diplobacillus associated with kidney disease is still uncertain. Thus, with our present knowledge, it is difficult or impossible to eradicate the malady from an infected hatchery.

Histopathologic studies were undertaken to clarify the pathology of the disease and to compare the eastern form with the western form.

Materials and Methods

Fish exhibiting the typical lesions of kidney disease were selected from two eastern and two western hatcheries which were having active epizootics. Yearling and fingerling brook trout were available from the Fish and Wildlife Service stations at Cortland, New York, and Berlin, New Hampshire. Yearling coho salmon were received from the Oregon Fish Commission station at Sandy, Oregon, and yearling fall chinook salmon from the Fish and Wildlife Service station at Underwood, Washington.

These fish were fixed in Bouin's solution for approximately 24 hours, then stored in 65 per cent alcohol. Paraffin sections were prepared from the tongue, eyes, brain, gill, heart, dorsal and ventral muscles, kidney, liver, gallbladder, air bladder, spleen, and from the digestive tract at seven equally divided regions. The sections were

* Received for publication, July 9, 1955.

stained with hematoxylin and eosin, Mallory's fungus stain, the Gram stain, and Wolbach's Giemsa variant.[6]

Histopathologic Findings

Grossly, the disease has been characterized as a systemic infection with a pronounced affinity for the kidney.[2,4] Belding and Merrill[1] suggested that the complex disease picture was due, in fact, to impaired renal function.

Histologically, the disease was exemplary of the chronic granulomas. Productive fibrotic lesions were observed in every organ of infected fish. The proliferating fibroblasts characteristically formed distinct nodules, but often large masses of tissue occurred without tubercle-like structures. The granulomatous lesions apparently arose in the connective tissue stroma between the parenchymal cells of the various organs. Elements of the reticulo-endothelial system were frequently involved. Giant cells occurred, but only infrequently. As the lesions enlarged, they encroached upon and overwhelmed the parenchymal tissues (Fig. 1). In terminal cases, destruction of essential organs was tremendous. It was, in fact, remarkable that fish survived to the point of such extensive tissue damage.

The first organ to be affected by a natural infection of kidney disease was not determined with certainty, but it was quite likely that the hematopoietic tissue of the kidney was first involved. (In fish, the kidney is the major site of blood production.) In the earliest stage observed in this study, the hematopoietic tissue was always in an advanced stage of destruction. As the disease progressed, the posterior portions of the kidney were obliterated by the massive tissue reaction which apparently outstripped the vascular supply and terminated in necrosis and suppuration. In the anterior parts of the kidney, the granuloma usually progressed in a more orderly fashion, resulting in extensive fibrotic lesions which gradually enveloped the included renal tissue (Fig. 4).

Subsequently, or perhaps simultaneously, certain changes occurred in the digestive tract. From the esophagus through the small intestine, a marked eosinophilic inflammation occurred in the muscular wall. Eosinophils normally are present in the submucosal layers of the intestine. In moribund fish, their numbers were increased (Fig. 2). In the large intestine, the inflammation was granulomatous and involved the subcutaneous tissues in an extensive cellular reaction. The mucosal folds had a solid core of proliferating fibroblastic tissue (Fig. 3). There was also a characteristic cellular proliferation around

the blood vessels of the intestinal tract, which was probably related to their endothelial elements.

The later stages of the intestinal lesions were characterized by an extensive peritonitis in the region of the gastric ceca. At first acute, with many inflammatory cells, both lymphocytic and polymorphonuclear, the lesions spread throughout the visceral fat and pancreatic tissue. The inflammation continued to the chronic granulomatous stage (Fig. 5), although sometimes it was replaced by massive necrosis affecting the entire pancreatic system (Fig. 6).

Cytoplasmic inclusion bodies were seen commonly in the pancreatic acinar cells. These inclusions did not resemble any described in the mammalian literature nor were they similar to those of infectious pancreatic necrosis.[7] They were round or ovoid, were often of tremendous size, forcing the nucleus to one side of the cell, and were strongly eosinophilic (Fig. 7). The larger forms sometimes contained basophilic globules or were uniformly basophilic (Fig. 8). The eosinophilic portions were gram-negative; the basophilic portions, gram-positive.

In the liver, granulomatous nodules formed in the connective tissue stroma between the cords of hepatic cells. These increased in size, forcing the liver tissue aside (Fig. 9). Actual necrosis of the parenchymal cells appeared minor but the hepatic tissue was gradually replaced by the proliferating inflammatory tissue. Cytoplasmic inclusions also were present in the parenchymal cells. These inclusions were most numerous at the periphery of necrotic areas (Figs. 10 and 11). When necrosis was absent, they were found adjacent to the granulomatous lesions in reduced numbers. Such inclusions were usually gram-negative, occasionally contained gram-positive globules, and were observed rarely with a high concentration of gram-positive diplobacilli. The liver inclusions appeared morphologically identical with the inclusions of the pancreatic acinar cells.

The splenic tissue was gradually replaced by the proliferating tissue of the granuloma until its normal structure was altered beyond recognition (Fig. 12).

As the disease entered the terminal stages, no tissue or organ escaped partial destruction. The heart was often the seat of a massive myocarditis with destruction of the major portion of the muscle (Fig. 13). The gills were enveloped in an inflammatory reaction extending from the arches to the tips of the filaments. The cerebral meninges usually contained complete granulomatous nodules (Fig. 14). The brain itself occasionally had necrotic foci or rings of inflammatory cells surround-

ing minor blood vessels (Fig. 15). The gallbladder and air bladder were sites of distinct granulomatous nodules. The subcutaneous tissue behind the eyeball was similarly affected.

The muscle lesions were distinctive. In most cases, they did not penetrate the epithelium of the skin and thus were not visible externally. They consisted of great masses of chronic inflammatory tissue extending far into the underlying muscular tissue (Fig. 16). The muscle lesions were a consistent site for the multinucleate giant cells so characteristic of the granulomas. In muscle sections, necrotic muscle bundles surrounded by normal tissue were frequently observed.

The cellular location of the gram-positive diplobacilli was of considerable interest. Both eastern and western workers have described the organism as being both intracellular and extracellular.[2,4] In tissue sections, the bacilli were rarely found concentrated in the parenchymal cells of any organ. In the liver and pancreas, they were found occasionally in parenchymal cells in close association with cytoplasmic inclusion bodies. In contrast, the organisms usually were found in tremendous numbers, both intracellularly and extracellularly, in the granulomatous lesions. In these areas, it appeared that the intracellular forms might be the result of active phagocytosis by inflammatory cells (Fig. 17). Many cells of the kidney were filled with the organisms. The lymphoid character of this tissue was also suggestive that phagocytosis was responsible for the intracellular position. It is entirely possible, however, that a specific intracellular stage was present in the cells of the granulomatous tissue. The areas of eosinophilic inflammation of the intestine did not contain the organisms although the muscular wall and mucosal folds were frequent sites for extracellular forms.

With Wolbach's Giemsa variant, the diplobacilli were stained reddish purple. Usually, the inclusions in the liver and pancreas were stained uniformly blue by this method. However, when many such inclusions were studied, there appeared to be a range of forms varying from homogeneous blue to packed masses of the violet diplobacilli with all intermediate stages.

Comparison Between the Eastern and Western Types

Histologically, the general character of the disease appeared nearly identical whether it occurred in western salmon or eastern trout. Occasionally, individual specimens were seen in which the acute phases of the disease had swept into massive necrosis with resulting death before the typical chronic stages of the granuloma developed. The disease

in salmon appeared to be somewhat more acute than it was in trout. The diplobacilli appeared morphologically identical regardless of the species of fish infected. In some of the trout examined, the organism stained less intensely with Gram and Giemsa stains and appeared smaller than the western form. Also in some trout, extensive granulomatous lesions were present when diplobacilli were nearly completely absent. These observations tend to confirm the reports of Snieszko and Griffin[4] who observed lesions of kidney disease without the concurrent presence of the diplobacilli.

The primary differences between the eastern and western forms of the disease appeared in connection with a secondary lesion which was rather characteristic. The eastern disease was often associated with a mycosis-like granuloma.[4,8] This, in effect, appeared to result in a second superimposed granuloma of different origin. The mycotic granuloma was sharply differentiated from the primary lesions by the frequent occurrence of foreign body giant cells in association with yeast-like organisms. In the western form of kidney disease, giant cells were limited almost entirely to the muscle lesions and the mycotic organism was not observed.

In the coho salmon from the Sandy, Oregon, hatchery, there was a marked incidence of the salmon poisoning fluke (*Nanophyetus salmincola* Chapin). The metacercariae were encysted in practically all of the fish tissues with heaviest concentrations in the posterior kidney and heart. Histologically, there was no apparent correlation between the encysted organisms and the lesions of kidney disease. In some cases the metacercariae were in the centers of granulomatous lesions. At other times, however, they were observed in tissues which were unaffected by kidney disease. The salmon poisoning fluke was not observed in the fall chinook salmon from Underwood, Washington, and was, of course, absent from the eastern samples.

A round, spherical organism, tentatively identified as *Schizamoeba salmonis*, was present in the anterior digestive tracts of both eastern and western samples. At the Cortland station, fish fed synthetic diets apparently were not infected by this organism but were infected with kidney disease. It would, therefore, appear doubtful that a significant relationship exists.

Discussion

The granulomatous character of the lesions of kidney disease indicates an organism of low virulence or, conversely, indicates a high degree of resistance by infected fish. Eastern workers have experienced difficulty in transmitting the disease systematically except by

injection.[4] This may be due to the apparent balance in pathogenicity of the organism versus the resistance of the host. It appears that salmon may be somewhat less resistant than trout, which may explain why western workers have less trouble in transmitting the disease by feeding infective materials.

The complex and extensive morphologic changes are probably not a direct result of impaired renal function. The renal system is one of the later tissues to be affected by the disease. Instead, each organ is affected directly by the pathogenic organism with the resulting symptoms. The edema frequently seen in the disease is probably the result of a damaged circulatory system, as is especially evident in the mesenteric blood vessels.

At present, the classification of the diplobacillus found in kidney disease is still in doubt. The rickettsia responsible for salmon poisoning in dogs[9] has been suggested as the causative agent of kidney disease.[2,4] The inclusions observed strongly indicate that an intracellular stage is present in the life cycle of the diplobacillus. However, Earp *et al.*[2] maintained at least limited growth of the organism on media containing coagulated chicken eggs, indicating that the organism may be bacterial instead of rickettsial. More recently, on the basis of slides from dogs,* Snieszko[10] believed there is little, if any, likelihood that the salmon poisoning of dogs is related to kidney disease. In mammals, granulomas are rarely attributed to rickettsial agents. Such diseases as typhus, spotted fever, Q fever, and trench fever, all rickettsial in origin, have practically nothing in common histopathologically with the lesions of kidney disease. The Bartonella group, members of the order Rickettsiales, do in some cases produce a granuloma and have, in addition, a marked affinity for the reticuloendothelial system. The diplobacillus is gram-positive, while the Rickettsia are gram-negative. The reactions of the two organisms with Giemsa's stain, however, are very similar. The question is not resolved and awaits further bacteriologic studies. The occurrence of inclusion bodies, their apparent relationship to the diplobacilli, and the presence of granulomatous lesions without diplobacilli also indicate that the disease is more complex than a simple systemic bacterial infection.

The significance of the secondary lesions is obscure. Several workers have suggested the salmon poisoning fluke as a carrier for the causative agent,[2,4] and Snieszko and Griffin[4] have theorized that there may be a correlation between incidence of the disease and concentration of

* Kindly furnished to S. F. Snieszko by W. J. Hadlow, D.V.M., National Institutes of Health, Hamilton, Montana.

carrier-parasites in the hatchery water supply. Since the salmon poisoning fluke has a very limited northwestern distribution, it is apparent that either it is not involved as a carrier or that more than one carrier exists. Aside from the host-parasite relationship, there is an additional likelihood that these supplementary pathogens are directly related to the incidence of kidney disease. Difficulty in transmitting the disease by contact and by feeding of infectious material is indicative of a high degree of resistance. It appears possible that these secondary pathogens may serve the important purpose of breaching the outer defenses of the fish and allowing entry to the disease organism. The possibility of such infection would be increased if the causative organism was present in other aquatic hosts. Brockway[11] has found morphologically similar, gram-positive diplobacilli in many aquatic animals, including the scud, salamander, mayfly, caddis fly, frog, chironimid, blood worm, and dragon fly larva. Even after the organism has gained access to the animal body, it apparently meets a strong resistance which culminates in death only after an extended period. The debilitating effect of the mycotic granuloma and of a massive infestation of the salmon poisoning fluke augments suspicion against these organisms even though they may be proved non-carriers of the kidney disease organism itself.

Summary

Histologically, the eastern and western forms of kidney disease are nearly identical. In both salmon and trout, the disease is characterized as a systemic granuloma.

Cytoplasmic inclusion bodies are present in the liver parenchymal cells and pancreatic acinar cells. These inclusions may be a stage in the life cycle of the causative agent.

The Gram-positive diplobacilli, which stain intensely with Giemsa's stain, are concentrated primarily in the granulomas where they occur both intracellularly and extracellularly. They are rarely seen in parenchymal cells.

The occurrence of secondary parasites in fish infected with kidney disease suggests that these parasites may have a rôle in introducing the causative agent and/or in lowering the resistance of the fish.

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LEGENDS FOR FIGURES

FIG. 1. Normal renal tubules surrounded by granulomatous tissue. Brook trout. Hematoxylin and eosin stain. × 200.

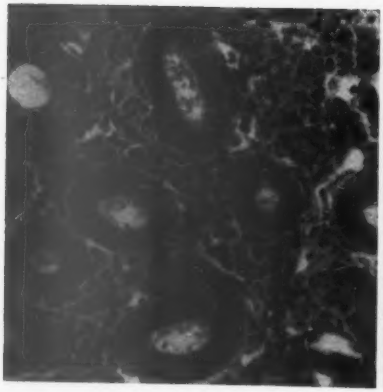
FIG. 2. Eosinophilic inflammation of the intestinal wall. Epithelium at top. Coho salmon. Hematoxylin and eosin stain. × 100.

FIG. 3. Granulomatous inflammation of large intestine. Brook trout. Hematoxylin and eosin stain. × 100.

FIG. 4. Necrosis and granuloma gradually engulfing the renal tubules. Coho salmon. Hematoxylin and eosin stain. × 100.

FIG. 5. Granulomatous tissue infiltrating the pancreas. Intestinal wall at lower left. Brook trout. Hematoxylin and eosin stain. × 100.

FIG. 6. Massive necrosis obliterating the pancreatic tissue. Gastric ceca at upper left. Chinook salmon. Hematoxylin and eosin stain. × 100.



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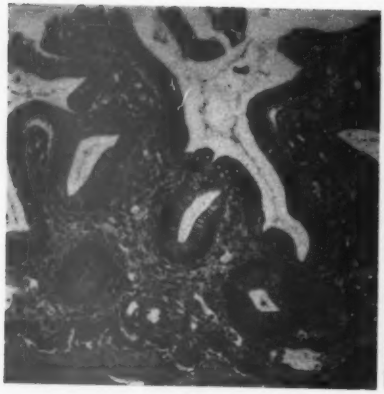

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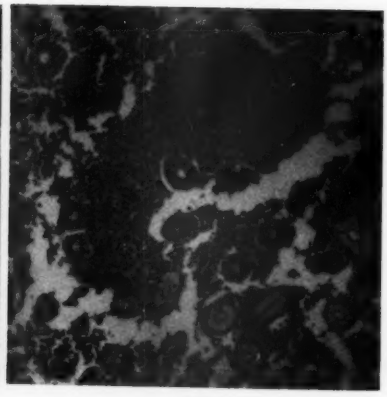

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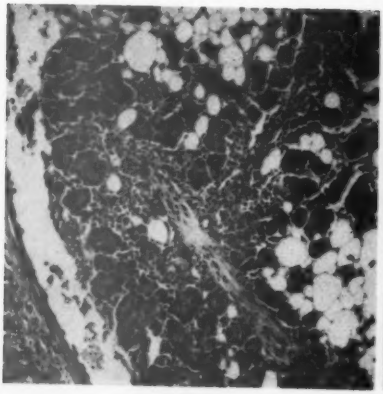

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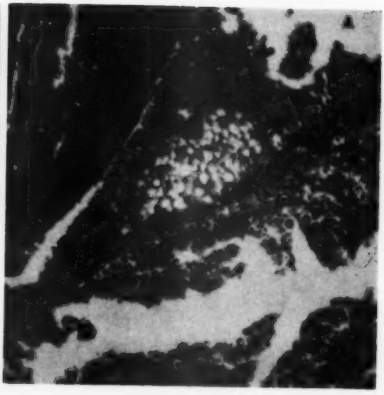

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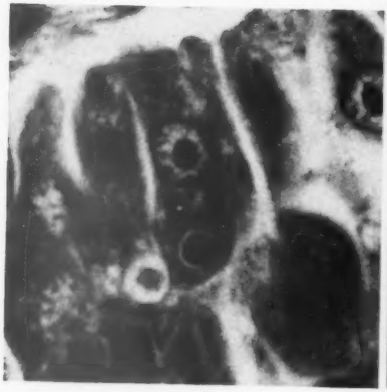
FIG. 7. An eosinophilic inclusion is present in the lower part of the pancreatic acinar cell in the center. Coho salmon. Hematoxylin and eosin stain. × 1350.

FIG. 8. From left to right, a lobed basophilic inclusion, an eosinophilic inclusion with basophilic granules, and an eosinophilic inclusion, all in pancreatic acinar cells. Coho salmon. Hematoxylin and eosin stain. × 1350.

FIG. 9. A granulomatous nodule, center, displacing the liver tissue. Brook trout. Hematoxylin and eosin stain. × 200.

FIGS. 10 and 11. Basophilic and eosinophilic inclusions in liver cells. Coho salmon. Hematoxylin and eosin stain. × 1350.

FIG. 12. The granulomatous proliferation has completely replaced the splenic tissue, making it impossible to discern the borderline between spleen and visceral fat. Brook trout. Hematoxylin and eosin stain. × 100.



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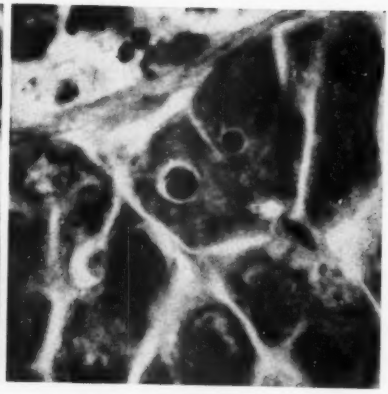

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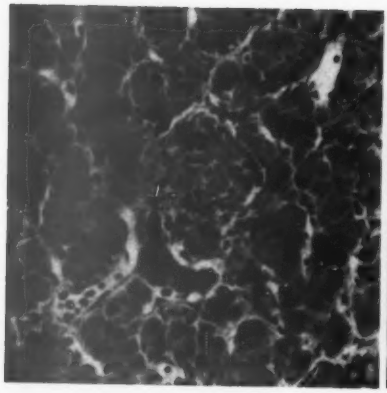

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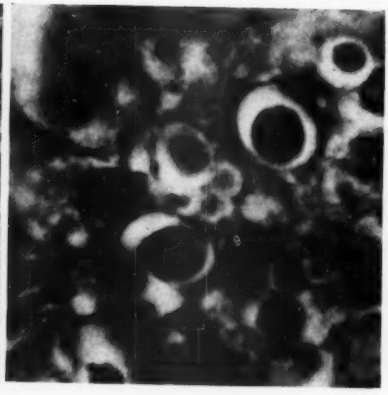

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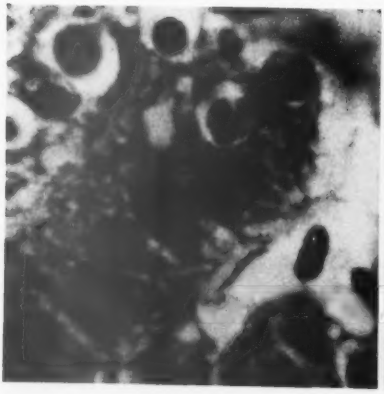

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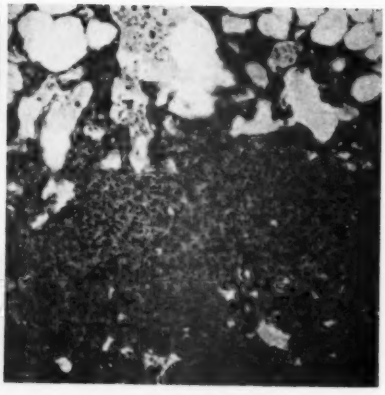

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FIG. 13. Granulomatous inflammation of the ventricle. Brook trout. Hematoxylin and eosin stain. × 100.

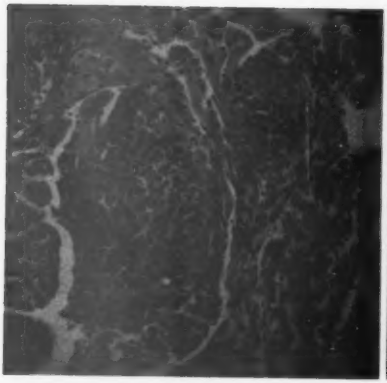
FIG. 14. Nodules of granulomatous tissue between the meninges and the brain proper. Chinook salmon. Hematoxylin and eosin stain. × 100.

FIG. 15. An inflammatory nodule and necrosis within the brain. Brook trout. Hematoxylin and eosin stain. × 100.

FIG. 16. The muscle lesion. Epithelium at top. Muscle myomeres are at right and left with the granulomatous lesion extending between them. Brook trout. Hematoxylin and eosin stain. × 60.

FIG. 17. Inflammatory cells filled with the diplobacilli, top center. Normal pancreatic acinar cells below. Coho salmon. Mallory's fungi stain. × 1350.

FIG. 18. Gram-positive diplobacilli. Brook trout. Gram stain. × 6000.



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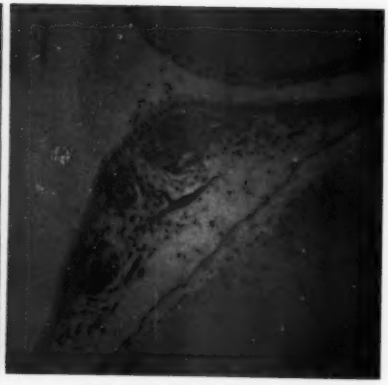

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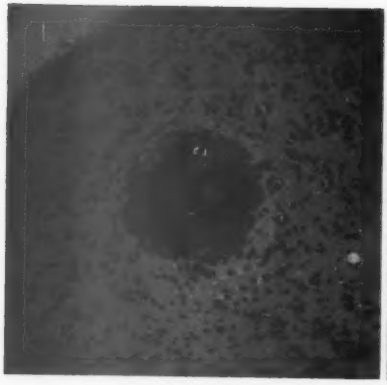

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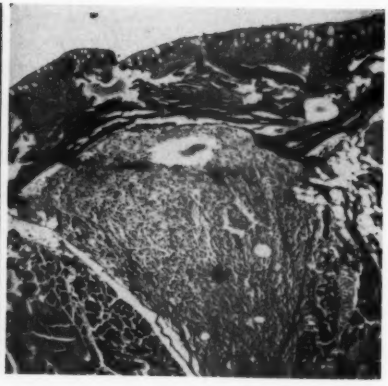

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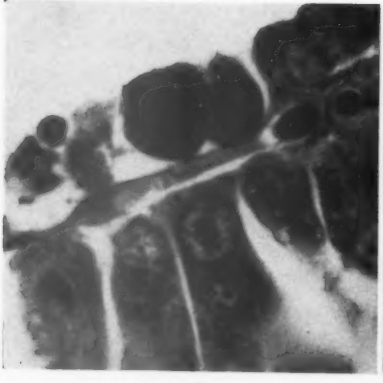

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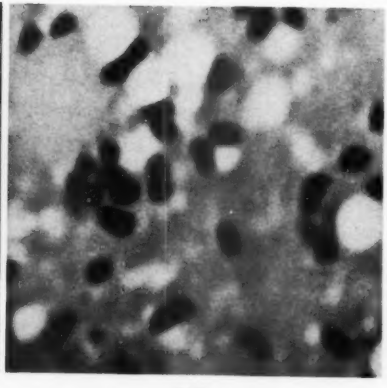

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THE EFFECT OF CORTISONE ON LOCALIZED INFLAMMATION IN THE LIVER OF RATS *

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In this article observations are described on the effect of cortisone on localized inflammation and repair in the livers of rats in which surgical gut has been implanted. Earlier studies[1,2] have indicated that this procedure provides the possibility of analyzing various effects on a reactive fibroplasia in the liver which is readily reproducible and which normally runs a fairly regular course for about 4 weeks from the date of operation. The present study seemed timely in view of conflicting evidence obtained from experiments on the effect of cortisone on the course of hepatic cirrhosis induced by carbon tetrachloride.[3-5]

MATERIAL AND METHODS

Plain surgical gut, size 3/0, was introduced into the median lobe of the livers of 52 male albino rats under ether anesthesia. The animals weighed 160 to 200 gm. at the time of operation, and were maintained on a standard laboratory diet.

In 16 rats cortisone acetate (Cortone, Merck) was administered subcutaneously in doses of 5 mg. per day, divided in two equal injections at an interval of 6 to 8 hours. Cortisone treatment began 2 days before implantation of the gut or immediately after laparotomy. Two to 5 rats in every group were examined at 2, 4, 7, 14, 16, and 26 days following operation. In a second series of 12 rats, administration of cortisone was commenced on the fourth day following implantation. The animals in this experiment were sacrificed at 7, 9, and 11 days; and at 2, 3, and 4 weeks.

At necropsy, two or three blocks were removed from the liver in the area of the gut and fixed in Zenker's acetic acid. Paraffin sections were stained with hematoxylin and eosin, and by Laidlaw's silver method, counterstained with van Gieson's mixture. The findings were compared with the reactive processes to implanted surgical gut in the livers of 24 untreated animals sacrificed at corresponding intervals.

* This investigation was supported in part by the Hadassah Medical Organization Research Fund.

Received for publication, November 8, 1955.

Results

Administration of Cortisone During the Early Period of Inflammation

The findings were similar irrespective of whether treatment was started immediately after operation or 2 days prior to it.

At 2 days following operation, the lesion was similar to that observed in untreated animals. The implanted gut was surrounded by a zone of necrotic liver tissue. A ring of abundant polymorphonuclear leukocytes was lying near the gut and invading the necrotic area (Fig. 1). A few young fibroblasts, concentrically arranged, were present adjacent to intact parenchyma in two of the treated rats.

After 4 days the necrotic liver tissue was absorbed and the area was cuffed by a narrow zone of mature collagen tissue, which contained hyperchromatic fibrocyte nuclei in small numbers and scattered histiocytes (Fig. 2). Silver impregnation revealed densely packed fibrils (Fig. 3). The surgical gut appeared unchanged. In control animals at this stage, the gut was surrounded by a wide zone of granulation tissue containing abundant fibroblasts but as yet poor in argyrophilic fibers (Fig. 4).

At *1 week* little further change had occurred in the gut or in the fibrous "sleeves" surrounding it, other than local hyaline change (Fig. 5) which progressed during the following week.

After *14 to 16 days* of treatment the fibrous ring surrounding the gut appeared further contracted (Fig. 6). Laidlaw's method revealed coarse reticulum fibers in abundance. The gut appeared to be intact. In untreated controls at this stage, the gut was invaded and surrounded by a ring of granulation tissue five or six times wider than in the cortisone treated rats. In one rat found dead on the 16th day of the experiment, the liver showed many abscesses; around the gut the lesions were similar to those described at 1 to 2 weeks following operation; in a third segment the gut was completely absorbed and replaced by almost acellular scar tissue with numerous capillaries.

At *26 days,* in one rat the gut was still present, encased in a thin capsule of collagen bundles: this liver contained numerous abscesses and wide areas of necrosis. In the second animal surviving to this stage, the area of implantation was not identified at necropsy and was missing in the histologic slides.

Administration of Cortisone Delayed Until the Fourth Day Following the Implantation

At *1 week* (corresponding to *3 days* of cortisone administration) the implanted gut appeared essentially preserved and surrounded by

poorly vascularized tissue. The tissue contained considerably fewer fibroblasts than in the controls, the nuclei being hyperchromatic and contracted and the collagen fibers in some areas were thickened and homogeneous (Fig. 7). The amount of fibrous tissue was larger than in both untreated controls and rats which had received cortisone from the time of laparotomy. Reticulum fibers were conglomerated and were much coarser than in the control animals (Fig. 8).

Nine, 11, and 14 days following operation (corresponding to up to 10 days of treatment with a total of 50 mg. of cortisone), the histologic picture was similar. The gut was fragmented and the pieces partially limited by shrunken histiocytes, and surrounded by a ring of fibrous tissue, poor in cells (Fig. 9). Here and there areas of hyalinosis were present, as were a few engorged capillaries. The reticulum fibers were abundant, coarse, irregular, and frequently conglomerated (Fig. 10). In untreated controls at 2 weeks, the implanted gut showed only moderate invasion by granulation tissue, which contained argyrophilic fibers well separated from one another and in lesser numbers than in the treated animals (Figs. 11 and 12).

After *3 and 4 weeks* almost identical lesions were found. The gut was completely absorbed and replaced by dense hyaline connective tissue containing sparse hyperchromatic fusiform nuclei and a few wide blood spaces lined by hypertrophic endothelial cells. With silver impregnation dense masses of reticulum fibers were seen (Fig. 13). At this stage the liver of untreated animals at the former site of the gut contained loose fibrous tissue with sparse argentaffin fibrils and marked infiltration with lymphoid elements (Fig. 14).

Discussion

When cortisone was administered from the beginning of the experiment, the acute response to the implanted gut was no different from that in untreated control animals. The first deviation from the normal course was seen after 4 days, reaffirming earlier observations of a lag of the cortisone effect in tissue repair during 1 to 4 days after the beginning of treatment.[6] At 4 days and later, suppression of the cellular and vascular components of granulation tissue was observed, identical with the changes revealed in tissues other than liver.[7-10]

A similar effect was observed also within 3 days when cortisone administration was started at the stage of maximal development of granulation tissue (4 days after implantation of surgical gut). This observation is not in agreement with earlier workers who concluded from studies on healing cutaneous wounds that granulation tissue, once formed, does not become modified by cortisone treatment.[11,12]

The fate of implanted gut composed of denatured collagen was different in cases with early and with delayed treatment. In the first group of animals the gut remained unabsorbed throughout the experiment. It is probable that absorption was impeded by the rapid maturation of scar tissue during the first week, which terminated the physiologic effect of the inflammatory process. An exception was seen in rats in which suppuration supervened in the liver as a result of accidental infection. That infection may alter the effect of cortisone has been reported previously by Lattes and coworkers.[13]

The complete absorption of the gut also proceeded normally when the administration of cortisone was delayed until after the completion of granulation tissue formation. It is likely that in such instances the gut was prepared for complete absorption by enzymes present in the granulation tissue. The leukocytic exudate seems to play a minor rôle in this process, since polymorphonuclear leukocytes were present in comparable numbers during the early stages of response, both in animals which had received cortisone for 2 days or from the time of implantation and in untreated controls in which absorption was completed by the third week.

The behavior of the fibrillar portion of the tissue reaction around the implanted gut during the later stages of repair deserves special attention. Despite the marked changes in quantity and appearance of the fibroblasts at 7 and 14 days, the scar tissue surrounding the gut was partially hyalinized and distinguished by agglomerated argyrophilic fibers, which were more abundant than in untreated controls. These findings were similar whether treatment with cortisone had been early or delayed. In the latter group, at 4 weeks and following the absorption of the gut, scars had formed which were somewhat larger and contained more prominent fibers than normally present.[1]

These findings are unusual in several respects. Firstly, we have observed previously that repair of acute damage to the liver caused only moderate production of scar tissue with spontaneous regression during a limited observation period of 2 to 3 weeks.[1,14] Moreover, experimental cirrhosis at various stages has been shown to regress spontaneously.[15,16] During the first 3 weeks of regeneration after subtotal hepatectomy, a definite delay of reticulum formation has been demonstrated by chemical methods.[17] On the other hand, in rats with carbon tetrachloride cirrhosis treated with cortisone, we have observed the reticulum fibers to be more abundant, coarse, and frequently agglomerated as compared with cirrhosis of untreated animals.[5]

Secondly, numerous studies of the effect of cortisone on different tissues have demonstrated that independently of the type of stimulation, whether by trauma, non-absorbable foreign body, or chemicals, suppression of granulation tissue is regularly accompanied by decreased formation of collagen fibers.[7,10] None of these observations, however, was made on hepatic tissue.

It appears, therefore, that the copious development and degeneration of reticulum fibers in repair processes in the liver are influenced by cortisone and this effect is characteristic for this site.

Summary

The effect of cortisone on the tissue response of the liver to implanted surgical gut has been investigated.

Administration of cortisone from the time of operation promoted faster maturation of scar tissue in the area surrounding the gut as compared with untreated controls. Cortisone also arrested absorption of the gut, except when accidental infection of the liver supervened.

Cortisone administration, when delayed until granulation tissue had been formed, caused rapid regressive changes in fibroblasts, but did not retard absorption of gut.

In both series of cortisone-treated animals the newly formed connective tissue showed hyaline change and more abundant argyrophilic fibrils than in the controls.

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LEGENDS FOR FIGURES

FIG. 1. Two days following operation. Leukocytic exudate between the implanted gut and adjacent necrotic liver tissue in a rat which received 5 mg. of cortisone daily for 4 days. Hematoxylin and eosin stain. × 560.

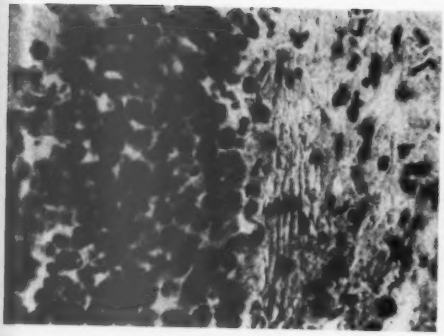
FIG. 2. Four days following operation; cortisone for 4 days. The gut is bordered by granulation tissue with hyperchromatic nuclei, frequently of bizarre appearance. Hematoxylin and eosin stain. × 560.

FIG. 3. From the same rat as that from which Figure 2 was taken. Laidlaw's method shows abundant argyrophilic fibers. × 560.

FIG. 4. Untreated control at 4 days. The gut is surrounded by active granulation tissue containing capillaries, histiocytes, and young fibroblasts. Hematoxylin and eosin stain. × 560.

FIG. 5. One week following implantation, the cortisone-treated rat shows gut surrounded by scar tissue with beginning hyalinization. Hematoxylin and eosin stain. × 560.

FIG. 6. At 2 weeks the scar around the gut appears further contracted. Hematoxylin and eosin stain. × 560.



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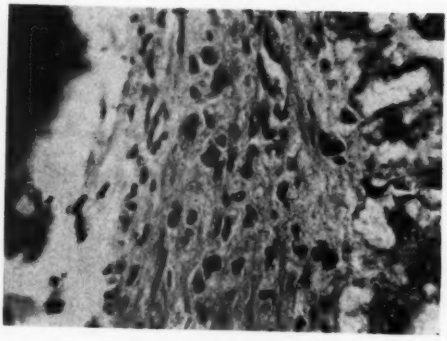

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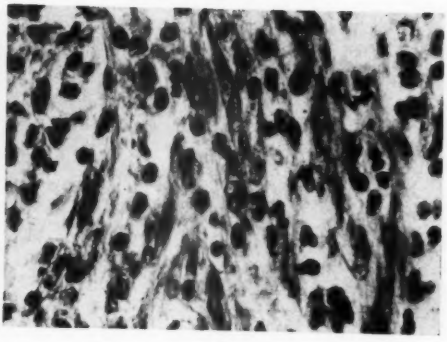

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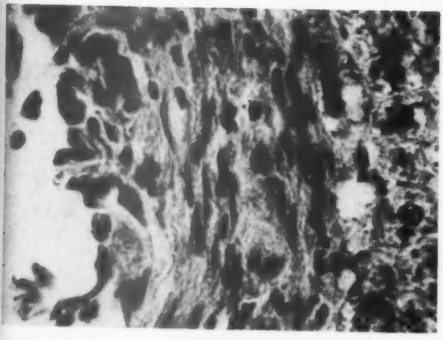

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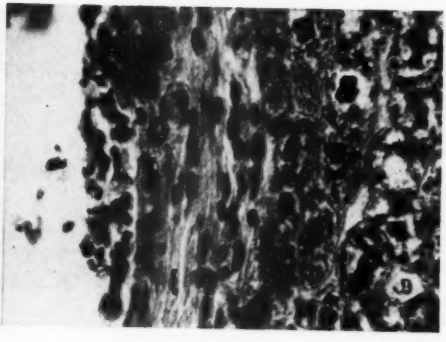

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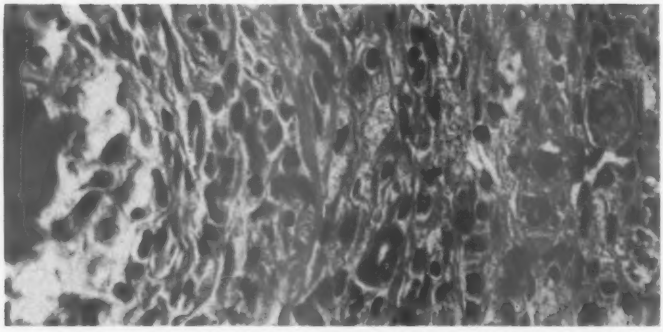

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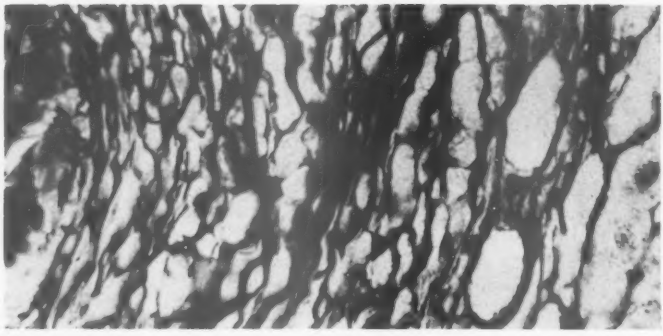

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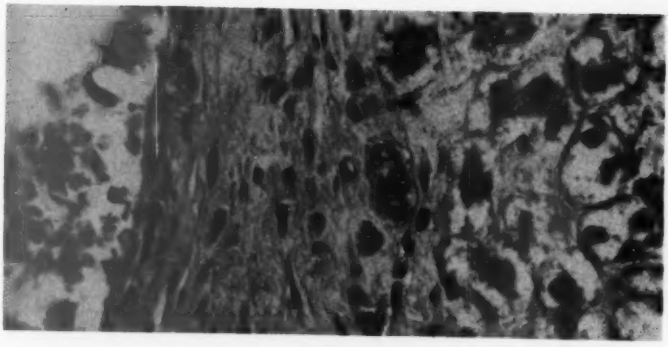
FIG. 7. Delayed administration of cortisone. One week following implantation of gut, after 3 days of administration of cortisone, total 15 mg. Regression of capillaries and of histiocytic infiltration. The fibroblastic nuclei are pyknotic and contracted. Collagen fibers are thickened. Hematoxylin and eosin stain. × 560.

FIG. 8. From the same rat as that from which Figure 7 was taken. Of note are coarse reticulum fibers. Laidlaw-van Gieson's stain. × 560.

FIG. 9. At 2 weeks and after 10 days of administration of cortisone, the gut is surrounded by a ring of mature scar tissue. Hematoxylin and eosin stain. × 560.

FIG. 10. The same area as shown in Figure 9. The fibrous area surrounding the gut contains conglomerations of thick argyrophilic fibers. Laidlaw-van Gieson's stain. × 560.

FIG. 11. Liver of untreated control rat, 2 weeks following implantation. Active granulation tissue, with beginning regression. Hematoxylin and eosin stain. × 560.



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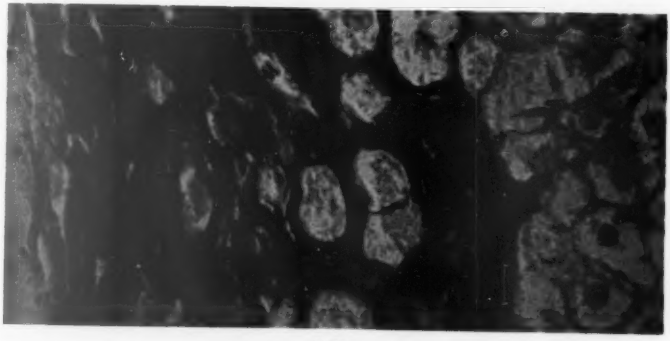

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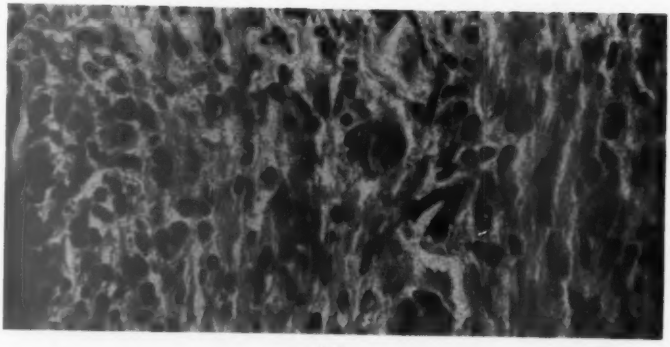

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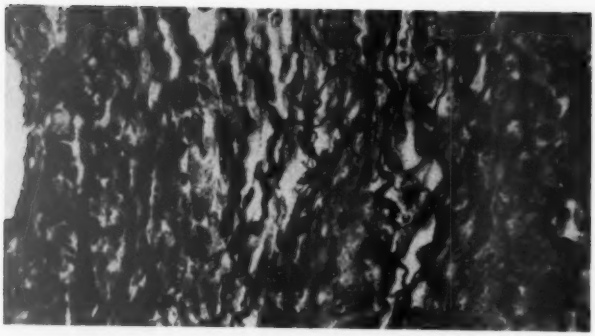
FIG. 12. From the same area as Figure 11. Isolated argyrophilic fibers of moderate thickness. Laidlaw-van Gieson's stain. × 560.

FIG. 13. Scar, 4 weeks after implantation; administration of cortisone for 24 days, beginning on the fourth day following operation. Laidlaw-van Gieson's stain. × 150.

FIG. 14. Scar at 4 weeks after implantation. Untreated control. Laidlaw-van Gieson's stain. × 150.



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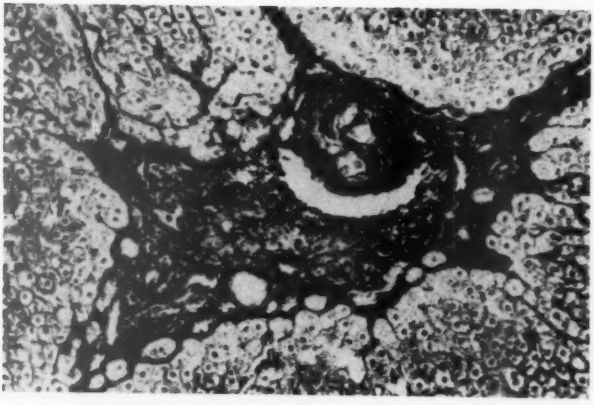

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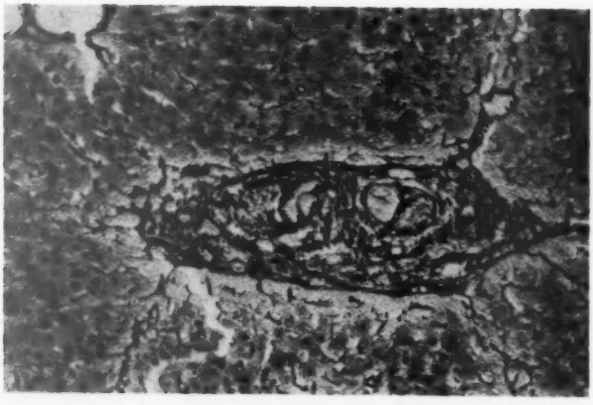

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